Swapping Mallards: Monocular imprints in ducklings are unavailable to the opposite eye

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Abstract

Eutherian mammals are unique in that sensory input from each eye is exchanged and shared between left and right brain hemispheres through the corpus callosum. All other vertebrates lack this structure and hence interocular information exchange is more restricted, raising issues of how information acquired with each eye contributes to the control of behaviour. Studies of food hoarding, laboratory discrimination, and homing in birds show that information acquired with one eye is not immediately available for action guided by the opposite one. We investigate interocular transfer, using filial imprinting in ducklings as experimental system. In Experiment 1 we imprinted hatchlings on either of two duck decoys, in three treatments differing on whether (A) birds were trained and later tested for a following response binocularly, (B) trained and tested monocularly, with the same eye, or (C) trained and tested monocularly, with opposite eyes. Birds preferred the training decoy for at least 3 hours after imprinting in treatments A and B, but were indifferent in C. In Experiment 2 birds were imprinted sequentially with two decoys, in three treatments where they were (D) trained and tested binocularly, (E) trained monocularly with a different decoy for each eye and tested monocularly with each eye, or (F) trained monocularly with a different decoy for each eye and tested binocularly. In treatment D ducklings were close to indifference, with a weak preference for the most recent decoy. In treatment E preference weakly favoured the decoy used during imprinting with the eye being tested. Finally, in treatment F there was no evidence for dominance of either eye. Thus, imprinting information is laterally isolated for at least three hours, the experience status of the opposite eye (naïve or with a competing imprinting) has a small effect, and we found no evidence for eye dominance.

Keywords: *Anas platyrhynchos,* attention, hemispheric integration, imprinting, laterality, mallard, pekin duck

Introduction

Among vertebrates, only eutherian mammals have a corpus callosum, the main anatomical structure allowing a high level of rapid information exchange between the left and right brain hemispheres. In the case of birds, whilst several smaller commissures connect the two hemispheres (Parsons and Rogers, 1993; Rogers et al., 2013), the relative independence (compared with mammals) of information processing between the left and right sides of the brain is interesting for a number of reasons. On one hand, relating differences in neuroanatomy to differences in behaviour between mammals and birds allows for deeper understanding of how action is controlled by the brain in general. On the other, it makes the avian bird a useful model for exploring hemispheric information integration, including how a system so different from the mammalian one controls attention and use of acquired information to control action.

The avian visual system consists primarily of the tectofugal and thalamofugal pathways. In most birds, the optic nerves are completely decussated, and the inputs from each eye project only to the contralateral hemisphere. From there, in both the tectofugal and thalamofugal pathways, inputs remain mostly within the same hemisphere: in the tectofugal, the right tectum projects to the right nucleus rotundus and *vice versa*, and in the thalamofugal, the right dorsal lateral geniculate nucleus projects to the right wulst and *vice versa*. In both pathways, there are also smaller interhemispheric projections, which are generally smaller then the ipsilateral projections, and in some species, lateralised, with one hemisphere sharing more information contralaterally than the other (Parsons and Rogers, 1993; Diekamp et al., 1999; Manns and Ströckens, 2014). This results in brains in which each hemisphere may have a better representation of the contralateral eye's inputs than the ipsilateral, and in which one hemisphere may have more ipsilateral eye information than the other.

Monocular learning experiments have demonstrated varying levels of hemispheric integration of visual information in bird species. Experiments in marsh tits (*Poecile palustris*) have shown that food cached with one eye open is not found when searching with the other (Sherry et al., 1981), but that information gathered with the left eye system is apparently transferred to long term memory in the right eye system between three and 24 hours following learning (Clayton, 1993). Incomplete contralateral visual input has been demonstrated in local area homing in pigeons (*Columba livia*) (Martinho et al., 2015), and interhemispheric transfer of pattern discrimination fails in chickens (*Gallus gallus*) (Gaston, 1979), though in the latter case, reinforcement with food enabled previously failed transfer (Gaston, 1984). This lack of complete access to the other hemisphere's visual memories has produced a similar pattern in binary discrimination and transitive inference tasks in pigeons (Diekamp et al., 1999; Manns and Römling, 2012). In most cases, visual input seems to be restricted to the eye-contralateral hemisphere, with integration via interhemispheric transfer occurring some time later. Several studies in young chicks have suggested that this hemispheric independence may support task specialization in each hemisphere, with left and right eyes and hemispheres taking on differing roles (Horn, 1979; Horn and Johnson, 1989; Deng and Rogers, 2002; Rogers et al., 2013).

Most existing studies dealing with hemispheric integration in birds have used adult birds and protracted learning, with the initial eye, to develop memory contents – a homing pigeon requires at least eight to ten flights over several days to develop a route, and binary choice and transitive inference require reinforcement over the course of many trials. The hoarding-retrieval experiments with adult marsh tits are an exception, because memory for the location of caches presumably takes place during the brief exposure to the hoarding site and its spatial context. Here we investigate interocular transfer in newly hatched ducklings, using imprinting as the critical task.

Avian filial imprinting is recognised as a unique form of learning – notable for having an obvious major biological function, occurring without explicit reinforcement, being fast, relatively permanent, and confined to well-defined critical or sensitive periods (Lorenz, 1937; Ramsay and Hess, 1954; Bateson, 1964; Bateson, 1966; Ratner and Hoffman, 1974; Bateson, 1979b). In its most characteristic natural form, imprinting manifests as a strong attraction by which young nidifugous birds tightly follow their mother (Bateson, 1966), her presence providing protection from predation, guidance to food sources, and in the case of the *Anatidae* waterfowl, waterproofing of the chicks' feathers until their own preen glands develop.

An imprint is formed by exposure to some stimulus during the sensitive period (Bateson, 1979b), and forms in a relatively short amount of time, with preference for the imprinted stimulus over a novel stimulus in a binary test occurring after as little as 15 minutes' of prior exposure to the imprinted stimulus (Bateson and Jaeckel, 1976). Imprints may be formed for multiple stimuli to which the chick is exposed during the sensitive period (Bolhuis and Bateson, 1990), and in such cases the multiple imprints are sensitive to primacy of formation and immediacy of exposure – more recently encountered stimuli are generally preferred to those encountered earlier, but equally recent exposure results in a preference for the imprint that formed earlier (Bolhuis and Bateson, 1990). Imprints can be formed of both visual and auditory stimuli (Boyd and Fabricius, 1965), and maternal auditory calls can enhance the strength of imprinted response to a visual stimulus (Dyer and Gottlieb, 1990).

Duckling imprinting is useful to investigate the autonomy of visual inputs and the time scale over which learned visual information may become available to the contralateral hemisphere, because multiple tests can be undertaken within minutes and hours of reliable acquisition, rather than after days of training.

Monocular imprinting has been investigated in ducklings before; Moltz and Stettner (1962) showed that a duckling imprinted monocularly on a moving duck decoy during several sessions would follow that decoy when only the contralateral eye was available (Moltz and Stettner, 1962). However, ducklings took several minutes to begin to follow the stimulus, and Moltz and Stettner rightly suggest that the following response exhibited during contralateral testing could be due to reacquisition of the imprint with the naïve eye, as the imprinted stimulus was the only moving object available to the duckling during testing (Moltz and Stettner, 1962). Furthermore, training occurred over three days, a span shown in other species to be sufficient for interhemispheric transfer, which may have allowed the ducklings access to interocular transfer not available immediately after initial imprinting.

We employ a protocol similar to that of Moltz and Stettner but include an additional, novel stimulus in the testing phase to compete with the imprinted stimulus, and confine all training to 30 minutes and all testing to within three hours of the conclusion of training. This allows us to rule out reacquisition with the naïve eye, because if imprinting begins anew with the contralateral eye in the testing phase, it would result in equivalent imprinting of the two decoys, regardless of the previous experience of the other (now occluded) eye. The very brief nature of imprint learning allows us to investigate whether information gathered with one eye is available to the other in the interval before interhemispheric transfer occurs (3 hours after acquisition, as in caching marsh tits). Furthermore, by testing at hourly intervals within this period, we probe the first hours after imprinting for evidence of interhemispheric transfer, which if present would result in increasing following fidelity to the originally imprinted stimulus.

Using moving imprinting stimuli shaped as adult duck decoys of indeterminate species, and identical to each other except for colour (see Figure 1), in Experiment 1 we investigate the capacity of ducklings imprinted with only one eye available to distinguish between an imprinted and novel stimulus at hourly intervals across the first three hours following learning. In Experiment 2, we explored inter-eye dominance and the influence of alternative eye experience by setting imprints formed with each eye at odds, allowing them to compete for control of the ducklings' preference.

Experimental Procedures

Experiment 1

Subjects

Subjects consisted of 48 newly hatched pekin (*Anas platyrhynchos domestica*) ducklings, from a variety of clutches within an established farm stock, between 15 and 31 hours old at the start of the experiments, divided into three groups of 16: binocular controls, monocular ipsilateral, and monocular contralateral. Ipsi- and contralateral refer to the relationship between the eyes used for imprinting and testing, as described below. Eggs were sourced from the Oxford University Farm in Oxford, UK, and incubated and hatched on-site. Incubation occurred largely in darkness, with occasional light exposure when eggs were moved between trays or moved to the hatcher.

Incubation and hatching

Eggs were incubated for approximately 28 days, and hatched in a dark hatching chamber to ensure that chicks formed no visual imprints prior to experimentation. Upon hatching, chicks remained in the dark chamber for approximately 24 hours to allow drying and to facilitate imprinting (Bolhuis and Bateson, 1990), as the highest sensitivity for imprinting has been shown to occur between thirteen and forty hours following hatching (Bateson, 1966). One hour before the commencement of experimentation, ducklings were moved to a brooding chamber illuminated from above with a 250W heat and light bulb suspended 50cm above the chamber, a protocol that has been reported as optimal for priming domestic chickens for high fidelity imprinting (Bateson and Wainwright, 1972). Monocular groups birds were fitted with occlusion goggles prior to being placed in the lighted chamber with *ad libitum* food and water (newly hatched ducklings eat very little as they consume their internal yolk). The goggles were designed to be swiftly secured and removed, loosely fitted around the head, and padded around the eyes to minimize any stress to the ducklings. Occlusion goggles were fashioned of elastic and frosted plastic and fastened with Velcro closures. The eye set to be available during imprinting was surrounded by an open ring, while the other eye was covered by a cone, ensuring no corneal contact. The conical lens allowed through a diffuse and uniform field of light, ensuring that only the unobstructed eye could form images. The edges of the lenses that came into contact with the duckling's head were lined with ottoman silk. Duckling selection, goggle fitting, and all subsequent handling of ducklings during experimentation were performed in near darkness, illuminated by a faint green light (Bateson, 1979a) to prevent the formation of extraneous visual imprints.

Imprinting

Ducklings were imprinted on one of two visual stimuli. Both stimuli consisted of a plastic decoy duck of undefined species that had been painted entirely blue or red. Ducklings show some spontaneous colour preference in imprinting, and we thus selected colours that have evoked similar levels of spontaneous preference in previous studies (Schaefer and Hess, 1959). The decoys differed in colour, but were identical in shape and size. They were suspended 5cm from the imprinting chamber's floor with invisible fishing line from a rotating boom powered by a motor, which caused them to travel in a one-metre diameter circle at a speed of approximately one revolution per 30 seconds. Movement of stimuli has been shown to elicit better imprinting in domestic chickens (Bateson, 1966) and it allows for the expression of preference in following, and not just proximity. The imprinting chamber was illuminated by a 100W incandescent light bulb (one metre above the chamber floor) and consisted of a square with 1.1m sides.

The imprinting phase took place immediately following priming in the lighted chamber. Each chick was transferred to the imprinting chamber and allowed to interact with its imprinted stimulus (IS) for 25 minutes. The other coloured decoy served later as the novel stimulus (NS), during preference testing.

Testing

The testing chamber consisted of a 3.5m diagonal hexagonal enclosure surrounded by white curtains. In the centre of the enclosure, both the imprinted and the novel stimuli were suspended 5cm from the chamber's floor by a rotating boom, and rotated in apposition in a circle of diameter 1.75m, at approximately 1 revolution per 30 seconds.

Ducklings were placed under an opaque dome in the centre of the enclosure, and testing commenced by lifting the dome from outside the enclosure. Each test lasted 10 minutes.

Ducklings' first test began immediately following the imprinting phase. Between each subsequent test, ducklings were placed in a dark brooding chamber with *ad libitum* food and water access. Ducklings were brooded in groups, for welfare considerations and to improve ducklings' imprinting responses (Lickliter and Gottlieb, 1985; Lickliter and Gottlieb, 1987).

Testing Sequence

Figure 2a shows the experimental protocol schematically.

Group A – Binocular control: Ducklings in this group had both eyes available throughout. The number of animals imprinted on the blue and red decoys were balanced. Following imprinting, each bird was tested four times for preference between the decoys. The first test commenced immediately after imprinting, and each subsequent test was performed after a subsequent 1 hour delay, providing four measures of preference between the decoys (T0, T1, T2, and T3).

Group B – Monocular ipsilateral: Ducklings in this group were imprinted and tested with only one eye available (the same for imprinting and testing). The number of animals starting with each eye and each decoy were balanced. The testing sequence was as for the binocular control.

Group C – Monocular contralateral: Ducklings in this group were imprinted with one eye and immediately tested for preference with the same eye (Ipsilateral Test (IT)) to corroborate whether an imprint comparable to that demonstrated by the monocular ipsilateral group had formed. Immediately following this, the goggles were reversed, and ducklings were tested for preference 4 times at hourly intervals as the other groups, but with only their naïve eye available. The number of animals with each eye and each decoy were balanced.

Scoring

All sessions were video recorded from above. The total numbers of positive (approach) and negative (avoidance) responses to the stimuli were recorded during imprinting (one stimulus) and testing (two stimuli). Negative responses were defined as either a movement directly away from a stimulus previously approached, or a movement dodging an approaching decoy. Positive responses were defined so as to control for different patterns of movement across ducklings – some tended to follow their imprinted stimulus in a smooth motion, while others made many short, rapid approaches interspersed with brief pauses. Discrete movements toward a decoy were counted as one positive response. When a duckling consistently followed a stimulus around its circular path, one positive response, and no more, was counted for each 90° of stimulus movement, regardless of whether this was composed of many small movements, or part of a single smooth movement around the circle. This ensured that a duckling moving smoothly around the circle, or making many tens of discrete movements in keeping with the stimulus around the circle were both counted as four positive responses.

Experiment 2

Motivation

The results of Experiment 1 show that in ducklings each of the two brain hemispheres are capable of forming and maintaining an imprint, and of showing a response independent of the contralateral hemisphere. The experiment contrasted trained versus naïve eyes. Experiment 2 was designed to investigate whether each hemisphere can maintain a separate imprint simultaneously, and whether such imprints interact when neither eye is naïve.

Subjects

Subjects consisted of 40 ducklings aged, sourced and hatched as in Experiment 1. They were split in three groups: Group D – Binocular (imprinted and tested binocularly, n=8), Group E – Monocular (imprinted and tested monocularly, n=16) and Group F – Eye Competition (imprinted monocularly and tested binocularly, n=16).

Imprinting

Pre-experimental treatment was as in Experiment 1. Prior to imprinting, birds in the Monocular and Eye Competition groups had goggles fitted over one eye. All ducklings were imprinted sequentially on one and then the other decoy for 25 minutes each, with order balanced within groups. The binocular group underwent both imprinting sessions with no goggles, while both the monocular and competition groups wore goggles over one and then the other eye for each session, balanced for order and decoy. The second imprinting session began approximately two minutes after the conclusion of the first, allowing goggles to be switched to the other eye and ducklings to be moved to the appropriate chamber. Following the second imprinting session, ducklings were placed in the dark brooder for 30 minutes.

Testing

Figure 2b shows the protocol graphically. Tests for preference were as in Experiment 1, except for the 30 minutes in the dark incubator between the second imprinting session and testing. Ducklings in the binocular and competition groups were tested once, with no goggles. Ducklings in the monocular group were tested twice in succession, once with each eye, with a two minute interval to allow switching the goggles to the other eye. Eye and decoy order were balanced. Tests were scored as in Experiment 1.

Ethical Note

These experiments were conducted according to the University of Oxford's Department of Zoology animal welfare standards. The experimental protocols were approved by the University of Oxford's Animal Welfare and Ethical Review Body. Eighty-eight one-to-two day old domesticated mallard ducklings of unknown sex were used in the experiment. The animals were incubated and hatched by Oxford University Farms and returned to their care upon completion of trials. Ducklings were housed together in a heated industrial brooding chamber before and after experiments and in smaller social brooding baskets with overhead heat during experimental intervals. Handling was kept to a minimum to avoid disturbance, and consisted only of moving animals from one chamber to another (which occurred over a matter of seconds), and of fitting goggles. The goggles were designed with Velcro release to allow swift fitting and removal (again, on the order of seconds) and were lined with silk to avoid discomfort. No invasive procedures occurred in this study.

Results

Experiment 1

The results of preference tests are displayed in Figure 3. Subjects in the Binocular and monocular ipsilateral groups showed a circa 70% preference for the imprinted stimulus. Birds in the monocular contralateral group showed a similar degree of preference for the imprinted stimulus in their ipsilateral test, but no significant preference between the imprinted and novel stimulus in the contralateral tests. Whilst birds testing with the right eye (regardless of group) showed higher fidelity preference for the imprinted stimulus than those tested with left, the effect of ipsilateral versus contralateral testing had a consistent effect on the strength of preference, regardless of eye. (See Figure S1, Supplementary Materials)

For our overall statistical analysis we excluded the ipsilateral test that was exclusive to the monocular contralateral group, performing a linear mixed effects analysis of the effects of group treatment on preference using the *lme4* package (Bates et al., 2014) in R (R Core Team, 2013). We used fixed effects of group treatment, test number (and therefore, time from imprinting), colour imprinted, and eye imprinted, and individual as random effect, and found through stepwise deletion by likelihood test no effect of colour imprinted or test number. (Colour preferences are shown in Figure S2, Supplementary Materials). Visual inspection of residual plots did not reveal any deviation from homoscedasticity or normality, and in spite of being a proportion, preference did not deviate significantly from a normal distribution in any of the preference tests (Kolmogorov-Smirnov test, $P > 0.63$ for all tests) nor did they differ in variance (Levene test, $P > 0.23$ for all tests), so no transformation was required. P-values were obtained by a likelihood ratio test of the full model with group treatment and eye imprinted against a null model without group treatment or eye imprinted. We found a significant effect of group treatment ($\chi^2_1 = 21.49$, $P < 0.001$) and of eye imprinted ($\chi^2_1 = 8.42$, $P = 0.004$) on preference. This analysis is conservative with respect to the existence of interocular transfer, because during the preference tests the birds were exposed to both decoys and this could influence the data towards neutrality, allowing ducklings to 'revise' their object of imprinting. The fact that the binocular and monocular ipsilateral groups still preferred the imprinted decoy after 3 hours, and the monocular contralateral group did not drift towards the original stimulus, is consistent with lack of transfer and conservative preference.

Experiment 2

The results of Experiment 1 show a bias in access to imprinting information towards the eye available during acquisition for at least a few hours, but also indicate that there may be differences in the strength of imprinting between the two eyes. Indeed, the significant effect of eye imprinted in our linear model together with visual inspection of the results split by the eye used in imprinting (Figure S1, Supplementary Material) suggests that imprinting with the right eye generates slightly stronger preference than with the left. Asymmetries in the degree of neuroanatomical decussation in the avian brain are well known (Skiba et al., 2000), including (in chickens) in an imprinting context (Rogers et al., 2013) This, together with this weak behavioural evidence, raises the question of eye dominance. To test for potential effects of eye dominance directly, we conducted a second experiment.

In Experiment 2, a critical Eye Competition group, consisting of sixteen ducklings, was, in the imprinting phase, exposed monocularly and sequentially (in different sessions) to a different decoy with each eye, and was then tested binocularly, to examine if there was a preference for the stimulus imprinted with either eye. Two other groups served to further investigate the results of Experiment 1. Individuals in the monocular group, also consisting of sixteen ducklings, were imprinted monocularly and sequentially with each of the two decoys in separate sessions, and then tested monocularly for preference with each eye in turn. In this group, the results of Experiment 1 would be confirmed by observing a preference for the decoy on which the eye being tested had been imprinted. Finally, the binocular group, consisting of eight ducklings, was imprinted on each decoy binocularly and sequentially in different sessions and tested also binocularly to provide a benchmark for normal imprinting. All experimental treatments are illustrated in Figure 2b, and the results are summarized in Figure 4.

The binocular group showed no significant reliable effects of recency, or preference for the more recently imprinted stimulus (t(7) = 1.498, *P* = 0.178, two-tailed, one sample t test), contrasting with the effect previously found in chickens when using multiple imprints in sequence (Bolhuis and Bateson, 1990). The competition group showed no statistically significant preference for the stimuli imprinted with either eye (t(14) = 1.281, *P* = 0.221, two-tailed, one sample t test), in contrast to the rightward bias seen in Experiment 1. These contradictory results do not allow for any firm conclusion regarding eye dominance in this context.

The monocular group was doubly imprinted and tested twice, once with each eye. The main difference with the monocular contralateral group in Experiment 1 is that in the latter the occluded eye had no previous experience, while in Experiment 2 both eyes had equal exposure to a different imprinting object. To interpret the results of the monocular group, it helps considering two extremes: if there were absolutely no interocular transfer, preference when tested with each eye should be as strong for the corresponding training stimulus as observed in Experiment 1, while if information was fully shared between the two sides, there should be no preference for either stimulus in either test. Results were intermediate. Preference for the corresponding decoy was 62% for the left and 61% for the right eye imprinted stimulus. Preference ratios were normally distributed (*P* > 0.15, Kolmogorov-Smirnov test). Combined, preference for the decoy used during acquisition with the eye available in testing was stronger but still not conventionally significant (t(27) = 1.916*, P* = 0.066) in a two-tailed, one sample t test (it could be argued that one-tailed tests would be more appropriate because we tested for strongly predicted expectations). This suggest a low, but not absent, level of interocular influence.

Discussion

In Experiment 1, ducklings in the two monocular groups were imprinted using one eye and tested monocularly with either the imprinted (monocular ipsilateral group) or the naïve (monocular contralateral) eye. They preferred the imprinted to the novel decoy when tested with the ipsilateral eye, but showed no clear preference when tested with the contralateral eye. Thus, the content of visual imprinting from one eye did not drive behaviour guided by a contralateral naïve eye, at least within three hours. This is probably due to lack of interhemispheric information transfer, and places the visual behaviour of newborn mallards within the model proposed for some other adult birds, including pigeons and chickens (Gaston, 1979; 1984; Diekamp et al., 1999; Manns and Römling, 2012; Martinho et al., 2015), and possibly marsh tits (Sherry et al., 1981; Clayton, 1993; Clayton and Krebs, 1994).

In the monocular groups of Experiment 2 neither eye was naïve, as each had been imprinted on a different decoy. In this case, when tested monocularly, preference for the ipsilaterally imprinted (and then predicted) decoy was weaker (ca. 61% rather than ca. 70%) and less statistically reliable than in Experiment 1 ($P < 0.07$ rather than $P < 0.001$, both 2-tailed). This between-experiments comparison is not direct evidence of an effect of state of knowledge in the contralateral eye (naïve in Experiment 1 and having a competing imprint in Experiment 2), but it indicates that it would be incautious to exclude the presence of some interocular interactions in the timescale explored, and is definitely worth further investigation.

In Experiment 1 the birds were tested monocularly for preference 4 times, at 1-hour intervals. During choice tests, the unblocked eye was exposed to both decoys, thus offering the possibility of new imprinting overwriting the original stimulus, causing a drift towards indifference, regardless of any potential inter-ocular transfer. Figure 3 does show that in the fourth trial, ducklings tested with their ipsilateral eye had a somewhat reduced bias, but testing for changes across the 3 hours did not yield a statistically reliable trend. At the same time, any transfer of information from the imprinted to the naïve eye would cause the opposite trend: ducklings tested contralaterally should shift from an initial neutrality towards preference for the originally exposed decoy, as the trained eye's information would influence the otherwise equal exposure to the two decoys by the naïve eye during testing. Figure 3 shows no detectable trend in this direction, and this was also reflected in the lack of any reliable interaction between preference and trial number.

Our results from the contralateral test in Experiment 1 stand in contrast to the previous monocular imprinting work in the same species by Moltz and Stettner (Moltz and Stettner, 1962). In particular, our monocularly imprinted ducklings' display of indifference when tested with the contralateral eye presented with two stimuli indicated the likelihood of the formation of new, possibly multiple imprints, when exposed to two stimuli with the naïve eye.

We did not detect reliable signs of dominance between the two eye systems. Experiment 2 addressed this directly by imprinting the ducklings monocularly twice, with a different decoy in each eye, and testing them binocularly. A strong dominance of one lateral visual system would lead to a preference for the decoy on which the dominant side was imprinted. The results however did not show any asymmetric effect. This may be partially attributable to the dark conditions during incubation. Studies in pigeons have shown asymmetry of hemispheric integration to be related to exposure of the eggs to light during incubation (Skiba et al., 2002; Manns and Römling, 2012), and it is possible that incubating ducks with greater exposure to light may induce such an effect in this species as well.

Conclusions

The relative independence between the two hemispheres of the avian brain has often been discussed and related to neuroanatomical asymmetries. The behavioural domains in which this has been explored include food caching and retrieving, navigation, and stimulus discrimination (Gaston, 1984; Clayton, 1993; Martinho et al., 2015). We now report such relative independence in filial imprinting, a form of learned behaviour that ontogenetically precedes the stage at which other animal systems were tested. Taken together, all such findings pose interesting problems for models dealing with the functioning of the avian brain and mind, including issues such as the potential multiplicity of simultaneous attention targets with each hemibrain.

It is tempting to speculate whether inter-hemispheric independence is an adaptation to some special property of the avian brain for the avian niche, or simply a reflection of the phylogenetically ancestral vertebrate architecture. Two arguments lead us to support the latter view. On one hand, retaining the independence of monocular visual input seems unlikely to be a very widespread need in the wild, although it may be true in some cases, such as when New Caledonian crows hunt with sticks for prey hidden in narrow, deep burrows (Troscianko et al., 2012; Martinho et al., 2014). In most ecological contexts both eyes will have a chance to be exposed to the contingencies to be learned, and hence laterally-acquired information will not dissociated as it has under experimental conditions. Our results, rather than calling for a post-hoc hypothesis for a functional advantage of keeping two non-integrated channels for visual information, suggest that birds manage differentiated visual inputs without a large hemispheric commissure akin to the mammalian *corpus callosum* through slower integration via the smaller alternative paths for inter-hemispheric communication that do exist in the avian brain. The effects of disruption of binocular vision are not exclusive to imprinting. They affect performance in caching (Clayton, 1993), transitive inference (Manns and Römling,

2012) and in homing route learning in pigeons (Martinho et al., 2015). On the other hand, the fact that only eutherian mammals possess a *corpus callosum* is extremely strong evidence that the absence of this structure is the ancestral condition, and hence its absence in young ducks is not in need of a functional explanation.

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Figure Legends

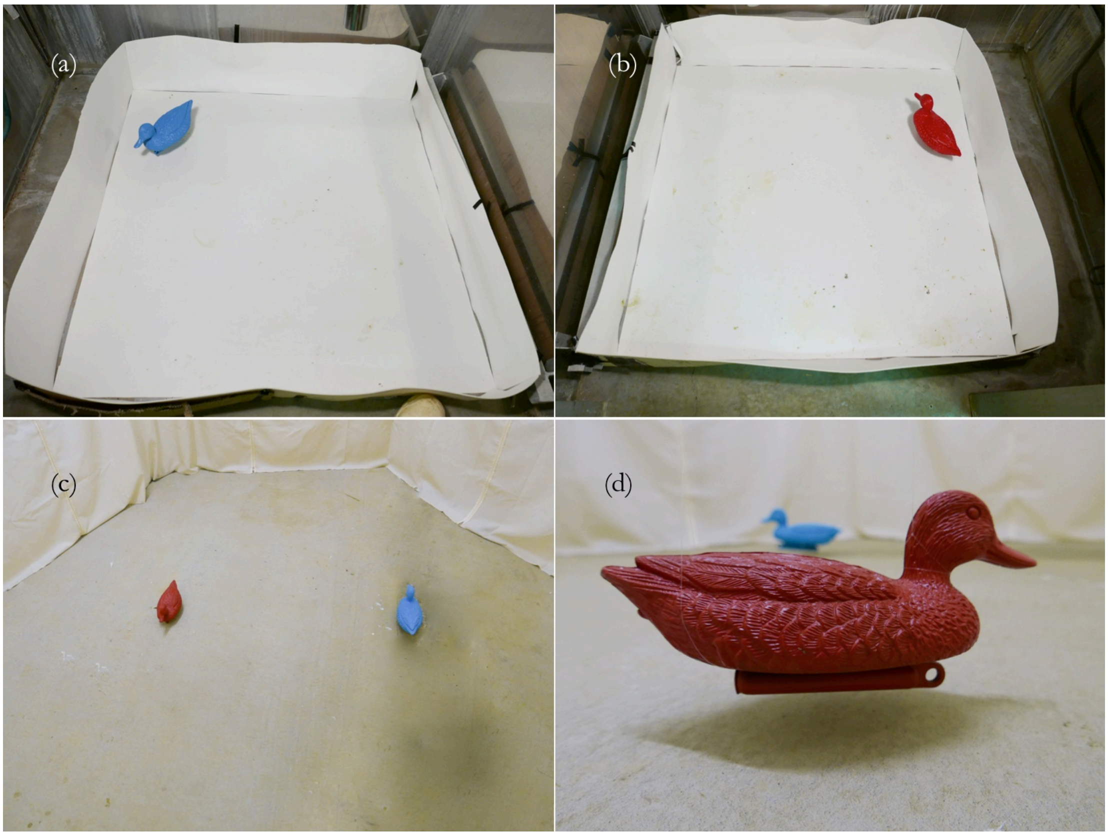

Figure 1. Training and Testing Chambers

(a,b) The training chambers contained a single moving stimulus, and consisted of a white square arena. (c) The testing chamber was a hexagonal arena within which two stimuli rotated about its centre. (d) Stimuli were suspended via invisible lines to hang approximately five centimeters above the floor.

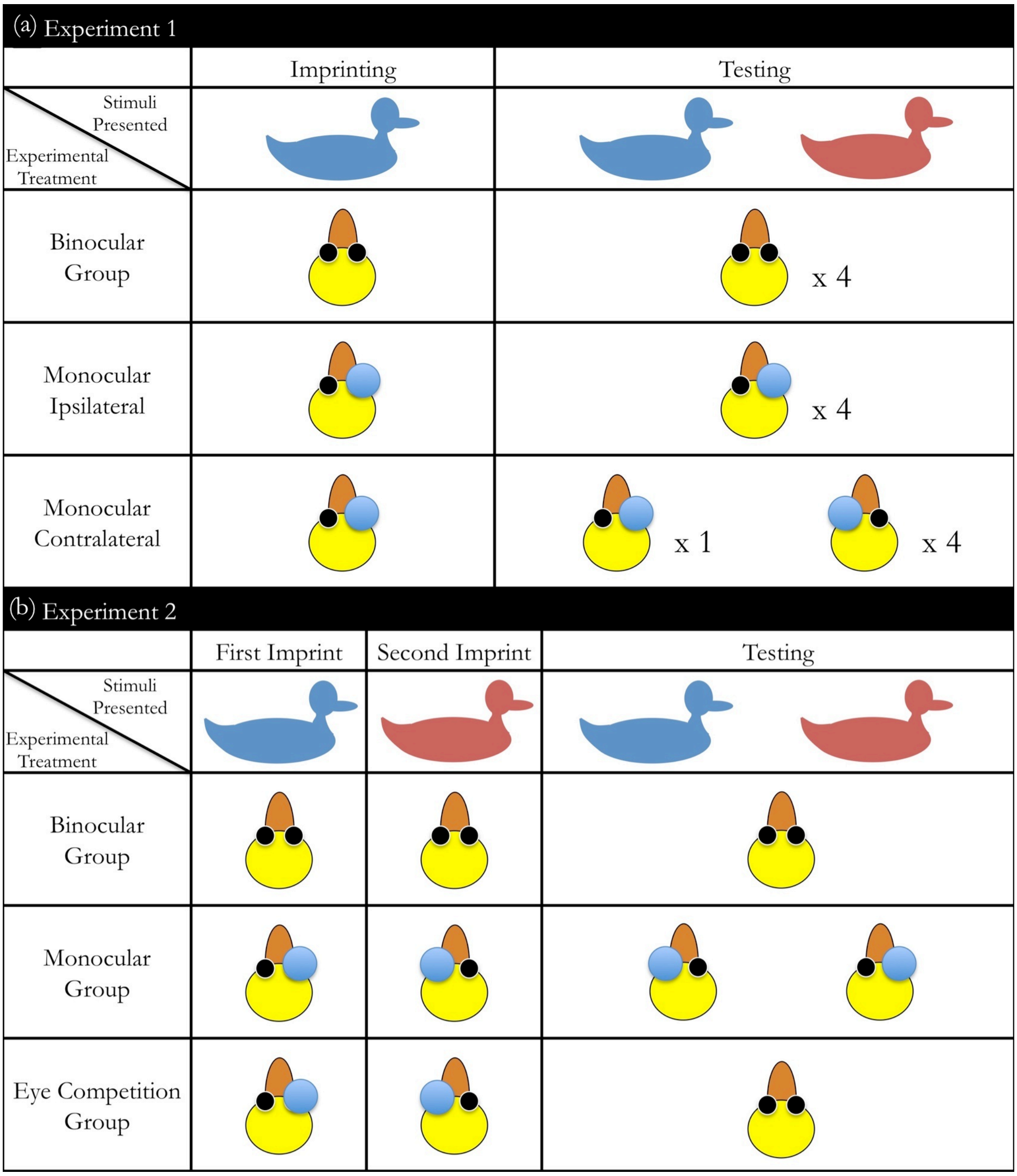

Figure 2. Schematic of Experimental Treatments

Sample schematic of imprinting and testing stimuli and treatments for each experiment and group. In all cases, one stimulus was presented in each imprinting phase, while both stimuli were presented in each preference test. (a) In Experiment 1 each duckling imprinted on one stimulus during the imprinting phase (the blue stimulus is shown here; groups were balanced between blue and red). In the binocular group, no occlusion goggles were used. The monocular ipsilateral group was occluded on the same side in both imprinting and testing. The monocular contralateral group was occluded on one side for imprinting and a single ‘ipsilateral test’, and then occluded on the other for the subsequent tests. In the monocular groups, half of the ducklings were occluded left and half right during imprinting, with subsequent occlusions as appropriate for the treatment. All birds performed four tests at hourly intervals following imprinting. The monocular contralateral group performed its ipsilateral test immediately between imprinting and the first contralateral test. (b) In Experiment 2, each duckling was imprinted on both stimuli in sequence (with sequence order balanced within groups). The binocular group wore no occlusion goggles in either imprinting phase. The monocular group and competition group were occluded on one side for the first imprint and the other for the second (balanced with respect to sequence). The binocular group and competition group were tested once, without occlusion. The monocular group was tested twice, once occluded on each side, balanced with respect to sequence.

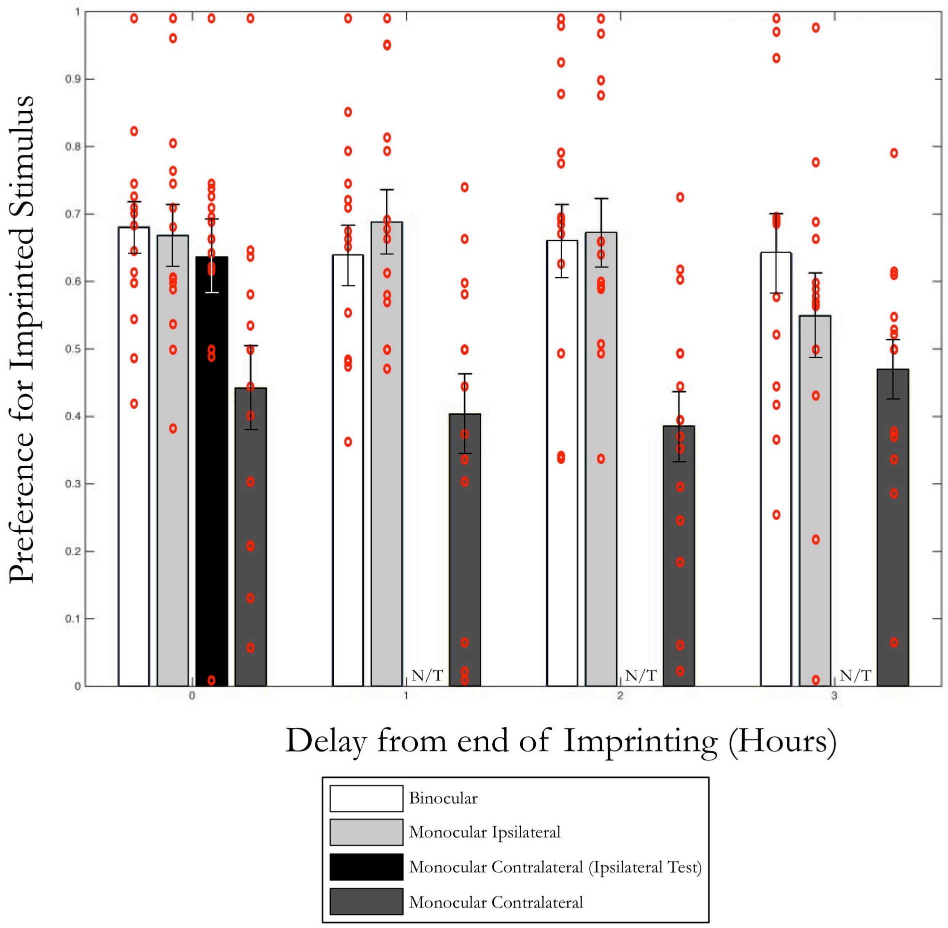

Figure 3. Stimulus preference (± standard error) during testing in Experiment 1. Binocular and monocular ipsilateral groups showed a significant preference for their Imprinted Stimulus during testing, as did monocular contralateral birds during their initial, ipsilateral test. The monocular contralateral group showed no significant preference during subsequent contralateral tests. Individual scores are shown as red rings. NB: The monocular contralateral birds were only tested ipsilaterally once, immediately preceding their 0 hour contralateral test, resulting in absent green bars (N/T, not tested) for time steps 1-3 hours.

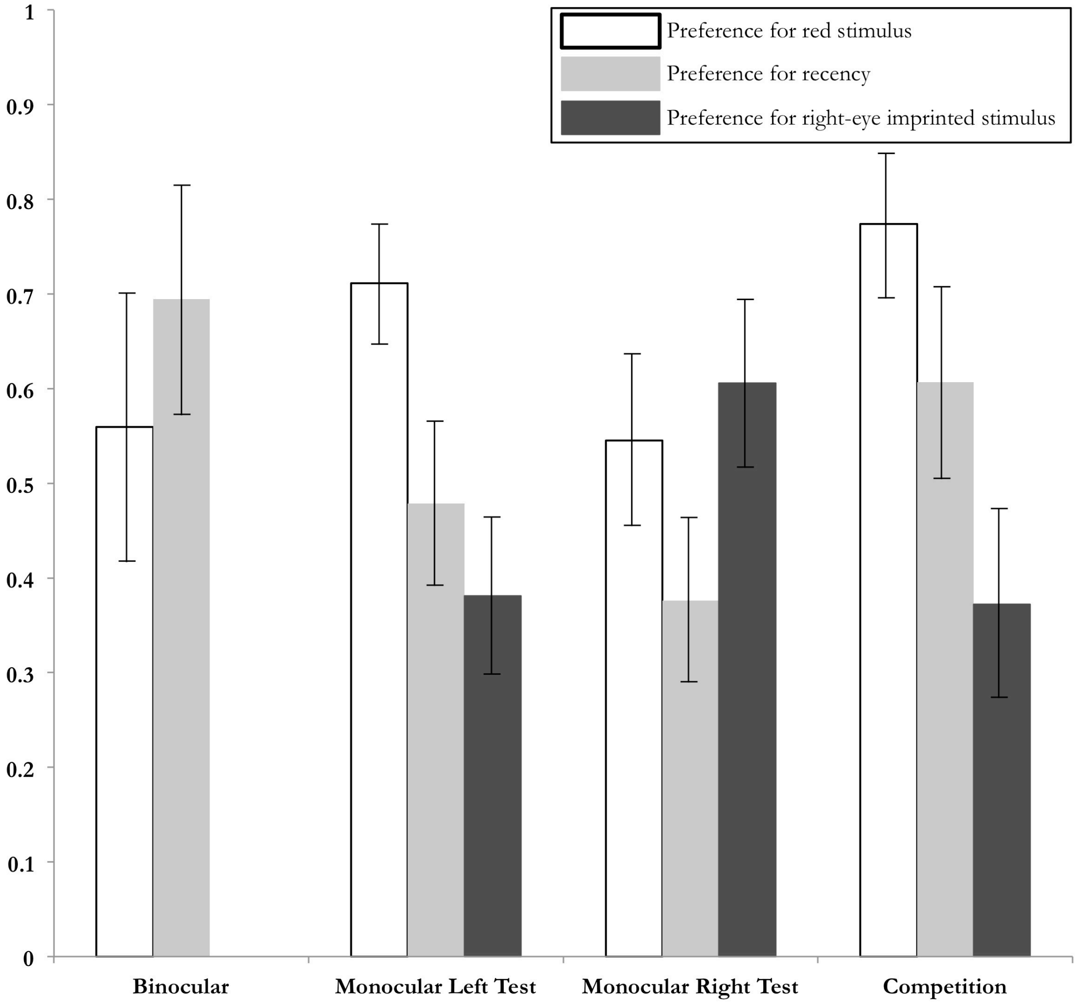

Figure 4. Stimulus preference (± standard error) during testing in Experiment 2. Mean preference is given for the red stimulus, the more recently imprinted stimulus, and the right-eye imprinted stimulus for each of the groups (twice for the Monocular Group, which was tested once with each eye).

Appendix – Supplementary Figures

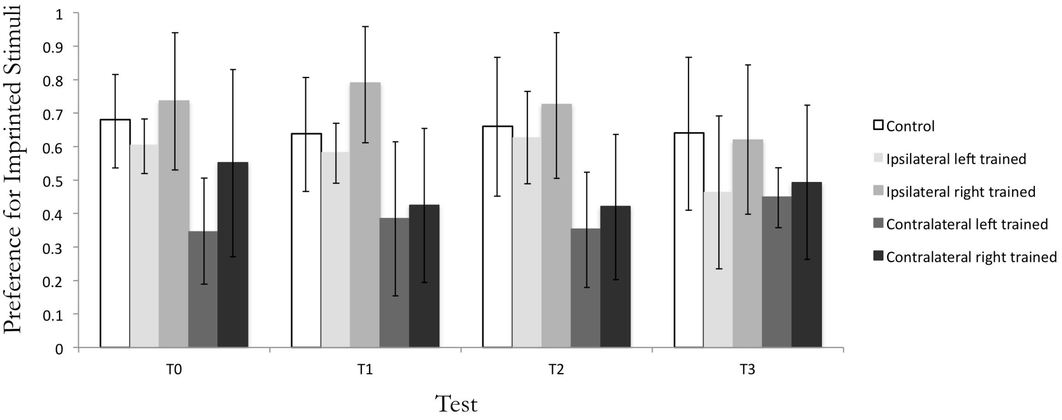

Figure S1. Stimulus Preference by eye in Experiment 1

The figure shows the average preference for the imprinted stimulus (as a proportion of total approaches to the imprinted and novel stimuli) during each test. Control and ipsilateral ducklings, regardless of which eye was trained, preferred the imprinted stimulus, while contralateral ducklings, again regardless of eye, showed no preference. Within the ipsilateral and contralateral groups, ducklings trained with the right eye showed a higher preference proportion than those trained with the left eye.

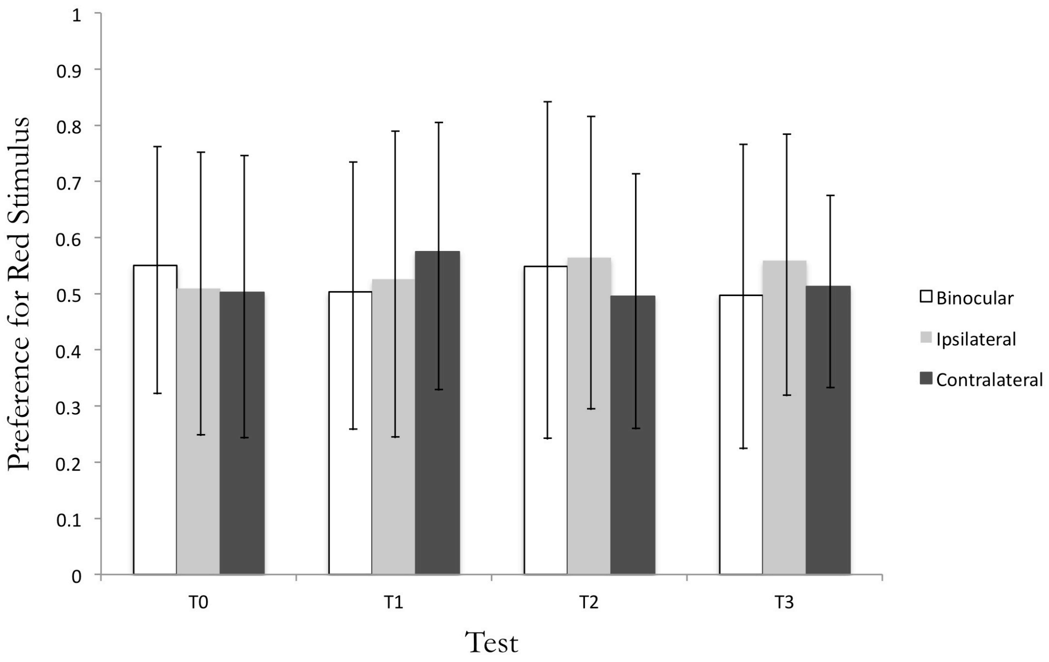

Figure S2. Stimulus Preference by colour in Experiment 1

The figure shows the average preference for the red stimulus (as a proportion of total approaches to the red and blue stimuli) during each test, regardless of which colour was imprinted. In all groups, there was no significant preference for one colour over the other, when both the blue-imprinted and the re-imprinted ducklings' test preferences were averaged together.

Animal Welfare

The experiments were conducted according to the University of Oxford Department of Zoology animal welfare standards and the experimental protocols were approved by the University of Oxford Animal Welfare and Ethical Review Body.

Data

Our data set is available on Mendeley Data at http://dx.doi.org/10.17632/djssy8yvz5.1

Author Contributions

The experiment was devised and carried out by AM with input and advice from AK. All trials were run by AM, and data analysis was carried out by AM with input from AK. Manuscript was prepared by AM and edited by AK.

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