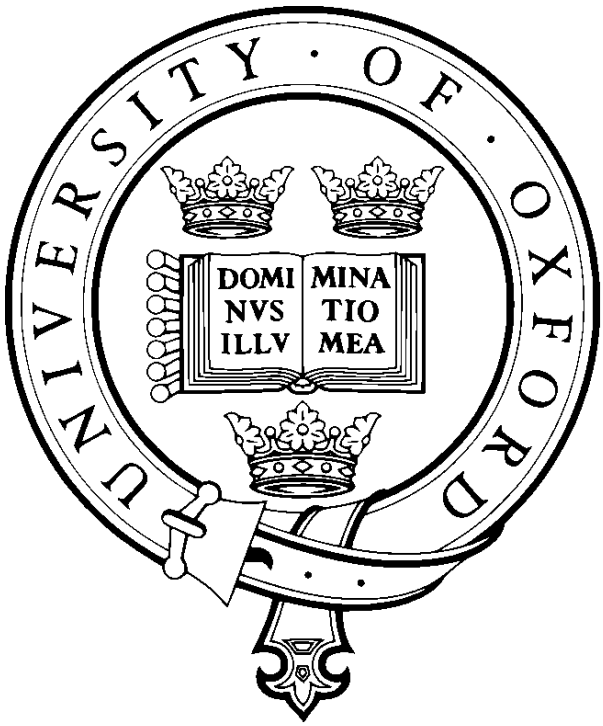
Ducks and Decussation: Information in the Avian Brain

Antone Martinho III

University College and the Department of Zoology

University of Oxford



Thesis submitted for the degree of Doctor of Philosophy

Trinity Term 2016

Abstract

A vertebrate brain must acquire, store, and process vast amounts of information. For birds, the majority of the incoming sensory information is visual, and like all vertebrates – except eutherian mammals – their visual system is bifurcated, with each eye feeding incoming information to the contralateral brain hemisphere. As a result, the bird must integrate these two banks of information to produce unified responses. This arrangement raises several questions about how a bird acquires, manages, and represents incoming visual information, and this thesis explores three of these questions. First, two studies investigate information integration across the two brain hemispheres in a long-term and short-term assay of birds trained monocularly with one eye and then tested with the other. The first finds that over a long term learning task in pigeons, some interhemispheric transfer of learned information occurs, but that complete integration does not occur. The second, using imprinting in ducklings, shows that the eye occluded during training has no demonstrable access to the learned information for at least three hours. A third study demonstrates that New Caledonian crows hold tools laterally in order to prioritize visual contact with the tool by their dominant eye during foraging, offering a potential underlying cause of this previously unexplained behavioural laterality. The fourth study explores the nature of information itself in the avian brain, showing that the maternal imprint formed by a newly hatched duckling can include abstract concepts defining the maternal stimulus, rather than just memory for visual images. This extends relational concept learning to a new species and suggests a much broader phylogenetic distribution of this ability. Combined, the studies suggest that the avian brain coordinates action managing two somewhat independent banks of information, biasing body positions in monocular tasks to allow control by a preferred eye, and has hitherto unsuspected capacity for abstract concept formation.

Acknowledgments

I am deeply indebted to so many whose hard work and support made this thesis and DPhil possible. First and foremost to my co-authors: each of my four data chapters represents a published or submitted paper with at least one co-author, and like all good science, none of these could have been achieved alone. Some of these co-authors are my supervisors, but others are colleagues and collaborators, and I thank Anna Gagliardo, Auguste von Bayern, and Zackory Burns for the tremendous part each played in making those studies possible. I extend a special thanks to Zackory, whose welcoming and informed introduction to Oxford, to the Department of Zoology, and to scientific publishing are a key stone in the foundation upon which this thesis rests.

Also co-authors, but more still – co-supervisors, are Dora Biro and Tim Guilford. Both have taken a tremendous role in guiding me towards this submission: editing manuscripts, vetting proposed experiments, teaching me to handle and work with pigeons, and welcoming me into the OxNav lab. On top of this, both were always at the ready with advice, support, and a reference in my seemingly endless job searches, and Tim has gone above and beyond the call of duty in helping learn to prepare and submit applications for new procedures to the Animal Welfare Ethical Review Body. I can never thank either enough for their part in this endeavour.

I am immensely grateful to Graham Taylor and Theresa Burt de Perera, my DPhil committee members for their support and advice throughout, and for always being ready to make sure my work was at its best: from sharp questions during my Transfer of Status viva to sharp questions in practice interviews and talks. Both of them have always been ready to lend an ear and a hand, and I am deeply indebted to them.

My colleagues in the "animal behaviour wing" of the Zoology Department have all contributed to making this thesis a success. My work has been hosted in both OxNav and the Behavioural Ecology Research Group, and all of the members of both have been a constant support network, as well as good friends. I am particularly grateful to Takao Sasaki, Izzy Watts, and Lucy Taylor in the pigeon lab for a wonderful working atmosphere and a flexibility and willingness to share our pigeon colony without which nothing could ever have gotten done – all three have been extremely patient and kind in training me in new methods, helping with statistics, working out rotas for use of the birds and equipment, and casting a critical eye over my work from time to time. On the Behavioural Ecology side, Andres Ojeda Laguna, Lauriane Rat-Fischer, and Stephen Barlow, my three office mates, have been constant sources of good conversation, good ideas, and better statistical and programming knowledge than I could hope to claim. I cannot count the number of times a problem that had stuck me for hours was answered in minutes, and without leaving my desk, thanks to the knowledge and helpfulness of these three.

No part of the research I have undertaken would have been possible without the constant efforts of the Zoology Department's skilled animal technicians, NACWO's, facilities team, and countless others. The vast network that makes the department run could fill pages of acknowledgements, but I extend particular gratitude to Phil Smith, David Wilson, and Lucy Larkman for their constant work to keep our animals healthy and our labs running. I also thank Mike Colley, poultry manager of the University Farm, for gracefully taking on the unexpected role of animal technician for the duckling experiments, and Mike Gooding, for helping us arrange that collaboration.

The Department's academic staff also deserve my special thanks for making the experience of working through this degree, from start to finish, entirely devoid of drama, difficulty, or unexpected hiccups – from the moment I arrived I have felt my academic feet on firm ground, and this is thanks especially to a team who have made themselves and their knowledge constantly available. I thank Heather Green for walking me through all the processes of an Oxford degree, from the first orientation, to Transfers, to Confirmation, and onward, as well as for lending a sympathetic ear whenever times got uncertain or difficult, and for a great round of tennis now and then. Adrian Thomas has played a tremendous role as well, both in his official capacity as Director of Graduate Studies and as a reliable advisor, practice interviewer, and referee, and I thank him for all of this and for providing me the healthfully distracting hobby of looking after, stocking, and lamenting the stocking problems of the C-floor aquarium. I extend as well a special thanks to him and to Sue Healy for accepting the arduous task of examining this thesis.

The entirety of University College, Oxford, deserves acknowledgement for providing the very ideal of a community and support structure in which to work toward submission. Beyond this, they have been so good as to employ me as Junior Dean, a role I have thoroughly enjoyed and which has relieved a great deal of the financial stress that comes with self-funding a DPhil. For this, and for wonderful new friends and mentors, I cannot thank the college enough. I am also thankful to Pembroke College, Oxford, for the opportunity to serve as Lecturer in Biological Sciences, wherein I have tremendously enjoyed learning to teach undergraduates. This degree would not have been possible without the financial support of the United States Federal Student Loans program, and I am grateful to my government and the policy makers who have prioritized education in this way.

My family, friends, and girlfriend have all provided constant help and support throughout the three years of my DPhil and of course in all my endeavours leading up to this point. My parents, Antone and Sandra Martinho, have constantly encouraged and supported me, wherever my interests have taken me, and I would not be submitting this thesis were it not for the sacrifices they have made to support me throughout. I am forever grateful to them for providing the kind of home where I could flourish, and hope I have done them proud. I am grateful to my brothers, Thomas and Trey Martinho, and all of my many friends at Oxford and beyond for always standing by to help me think or keep me distracted, as the case may require. My girlfriend, Emma Truswell, has had an enormous and unsung role in making this thesis possible. Always ready with a word of support, sharp edits of my sometimes-unnecessary verbosity, and help with experimental trials and conceptualizing results. She has always shown an interest and offered her ears and mind to help me refine ideas. I am immensely grateful to her.

Finally, and crucially, no part of this thesis could have occurred without the incredible efforts of Alex Kacelnik. My head supervisor, Alex has put countless hours into every part of my DPhil. He has always been available and ready to patiently field as many questions as I might have, to offer advice on experimental design, edit manuscripts, co-write grants, help prepare presentations, and even hold pigeons whilst I attached their goggles. But Alex has gone above the normal call of duty for a doctoral supervisor – he soldiered on through seventeen successive Junior Dean reference letters until I got one of the positions, supported me in job application after job application, secured weeks of housing for me in his college when I fell on tight times, and most importantly, hardly ever missed "prayer time" – our daily 11 AM coffee break. Alex has brought the art of supervising doctoral students to its apotheosis, and I am incredibly grateful to have been so privileged as to learn from him.

Author's Contribution

Regarding the thesis of Antone Martinho III, submitted for the degree of Doctor of Philosophy, Trinity Term 2016, entitled 'Ducks and Decussation: Information in the Avian Brain.'

All chapters have been developed, proofread, and edited by supervisors Alex Kacelnik, Dora Biro, and Tim Guilford.

Chapter 1. Introduction. Entirely my own work.

Chapter 2. Asymmetric Visual Input and Route Recapitulation in Homing Pigeons. This chapter is presented as a published manuscript. The experimental design, data collection, and analysis are my own work, with a small amount of data collection undertaken by two students I supervised. I wrote the published manuscript, which was edited and modified in collaboration with co-authors Dora Biro, Tim Guilford, Anna Gagliardo, and Alex Kacelnik.

Chapter 3. Swapping Mallards: Monocular Imprinting in Ducklings. This chapter is presented as a manuscript under consideration for publication. The experimental design, data collection, and analysis are my own work. I wrote the manuscript, which was edited and modified in collaboration with co-author Alex Kacelnik.

Chapter 4. Monocular tool control, eye dominance, and laterality in New Caledonian crows. This chapter is presented as a published manuscript. The experiment was devised by Zackory Burns and I in collaboration, with guidance from Auguste von Bayern and Alex Kacelnik. The data collection and analysis were primarily my own work, with assistance from Zackory Burns. I wrote the published manuscript, which was edited and modified in collaboration with co-authors Zackory Burns, Auguste von Bayern, and Alex Kacelnik. As the majority portion of the work was my own, the publication appears as an appendix to Zackory Burns' doctoral thesis, and as a primary chapter in my own.

Chapter 5. Same or Different?: Ducks Imprint on a Relational Concept. This chapter is presented as a published manuscript. The experimental design, data collection, and analysis are my own work, with video re-coded for accuracy by a student I supervised. I wrote the manuscript, which was edited and modified in collaboration with co-author Alex Kacelnik.

Chapter 6. Concluding Remarks. Entirely my own work.

TABLE OF CONTENTS

Chapter 1

Introduction

Antone Martinho III

Unpublished manuscript

"I warn you, if you bore me, I shall take my revenge."

J. R. R. Tolkien

Animals and Information

Sensory information comprises enormous amounts of data that need to be collected, connected, and stored to be of use to an animal moving within its environment. Vision in particular involves gathering vast quantities of information potentially critical to the success and survival of an animal.[1] Access to visual information and the accurate recall thereof is a critical component of behaviours that allow animals to survive, from food gathering, to movement, to conspecific identification. Birds, most of which are diurnal, are generally reliant on visual information to a greater degree than most mammals,[2] and the ways they obtain, store, and use information underpin their individual adaptive success.

To manage the inherently complex computational problem of all of this information, birds have at their disposal a brain very different from that of other vertebrates. Their brains are large, especially for a non-mammal, with respect to body size – this partly reflective in itself of the extensive visual input they process. However, birds are dinosaurs, and the neurological architecture of the avian brain reflects their divergence from the lineage of mammals over 300 million years ago, in many ways resembling the reptilian brain. Yet birds display abilities for intelligence and learning similar to and in some cases better than many mammals. The convergent evolution of these abilities marks them as a central topic in today's comparative cognition research.

This thesis seeks to understand how birds gather, store, and represent the visual information that dictates their survival success. The question is approached from three perspectives and across three avian species. The first section, comprising chapters two and three, explores how the retention of learned information and selective availability thereof to the two hemispheres of the brain affects behaviour in pigeons and mallards. The second section, chapter four,

involves work with New Caledonian crows investigating tool-holding laterality, an adaptation that may allow the crows to ensure consistent information by keeping dominant eye visual contact with the tool. The final section, again in mallards, explores how information is represented to ensure accurate stimulus identification, and proposes a much more robust conceptual capacity than previously ascribed to birds.

This program of research rests on a wide-ranging corpus of established knowledge on avian brain laterality and behavioural laterality, as well as taxonomically widespread work into the representation of information as perceptual and relational concepts. Here, I introduce these topics in detail and offer context to the four published or submitted manuscripts comprising the experimental chapters of this thesis.

On the Advantages of Being a Cyclops

Birds, as it happens, have two eyes; and binocular vision is an adaptation of many benefits – the relative lack of monocular organisms beside the taxonomic ubiquity of binocularity attests this. With two eyes, the field of vision is expanded,[3] stereoscopy is possible, perhaps contributing to depth perception,[4-6] and an animal is afforded with a degree of redundancy, both to ocular injury and also in that the binocular visual field allows two different views of objects within it.[7]

Having two eyes also presents an obvious problem, namely that two streams of visual information, from two distinct organs, must be integrated and resolved by the brain, a task complicated by the hemispheric division of the brain.[8,9] This problem has different solutions between eutherian mammals and birds. In the former, the optic nerves crossing and mixing at the chiasm allow

visual input to both hemispheres of the brain from each eye, and integration is further assisted by the presence of the *corpus callosum,*[10] the broad bundle of nerves connecting the two hemispheres and allowing relatively uninhibited transfer of information between the two halves of the brain.

In birds – and indeed in marsupials, monotremes, reptiles, amphibians, and fish – information sharing is more circuitous. In most birds, the optic chiasm is completely decussated:[11] the optic nerve from the left eye solely innervates the right brain hemisphere, and vice versa.[9,12] Furthermore, birds lack a *corpus callosum*; rather than a broad bridge of information transfer, the avian brain has several smaller hemispheric commissures, and transfer is limited.[9,12-14] This arrangement has inspired a field of research into how readily and how completely birds are able to integrate complementary information incoming via each eye. This is further complicated by the fact that the degree of interhemispheric integration may differ across species,[8] and in many cases, is lateralized, with one eye's inputs more completely shared to both hemispheres than the other's.[13,15]

A Thing of Twists and Turns

The avian visual system has two main input pathways, the tectofugal pathway and thalamofugal pathway.[9,12,16] These have been extensively studied, especially in the domestic chicken (*Gallus gallus*)[17] and the rock dove, or pigeon (*Columba livia*).[18] Even between these two species, there are neurological differences in how the two visual streams integrate. Both pathways do provide for ipsilateral input (that is, input from an eye to the brain hemisphere on the same side, in contrast to the optic nerves' decussation) via commisures,[19] but to varying degrees in each species, respectively.[20]

In pigeons, the thalamofugal pathway is balanced – the completely decussated inputs from each eye go to the contralateral brain hemisphere, which then sends a small input back to the ipsilateral side, roughly equal between sides. In chickens however, the thalamofugal pathway is lateralized: projections from each eye are exclusively to the contralateral *nucleus geniculatus lateralis pars dorsalis* (Gld), which in turn projects mainly to its co-lateral *wulst* (also contralateral to the input eye); each also projects more weakly to the opposite *wulst* (ipsilateral to the input eye). In contrast to the balanced inputs in the pigeon, however, the left Gld (with inputs from the right eye) projects to the right side of the brain much more strongly than the right Gld does to the left side. In pigeons, this system is balanced – strong inputs from each eye to the contralateral *wulst*, and equally weaker inputs to the ipsilateral *wulst*.[20]

The tectofugal pathway, however, is lateralized in both species, and in the opposite direction. In both pigeons and chickens, each eye projects exclusively to the contralateral optic *tectum*, which then projects mainly to its co-lateral *nucleus rotundus* and *entopallium*.[9,12,16,19] As in the thalamofugal pathway, the *tecta* also project contralaterally, but here it is the right *tectum* that projects to the left *nucleus rotundus* much more strongly than vice versa. While chickens have thalamofugal and tectofugal pathways lateralized opposite each other, pigeons are left lopsided – with a balanced thalamofugal pathway, but a tectofugal pathway that results in a left hemisphere with robust inputs from both eyes, but a right hemisphere that, based solely on the neuroanatomy, would seem to have access primarily to the inputs from the left eye.[20] (Figure 1 in Chapter 2 shows a schematic of the pigeon tectofugal system).

This asymmetry in the pigeon brain appears to be the result of the naturally asymmetric exposure of the developing eyes to external light permeating the egg

during incubation. Whilst still in the egg, the body of a developing bird is contorted in such a way that the right eye faces outward, exposed to diffuse light, whilst the left eye faces inwards and is occluded from this stimulus.[21-23] As altricial birds, pigeons undergo visual system development primarily after hatching – whilst the maturation of the tectofugal system begins during the final days of incubation, when light inputs are thus unbalanced, the thalamofugal pathway develops after hatching, when light stimulation to the two eyes is identical. Chickens, on the other hand, are precocial, and the near complete maturation of both systems occurs prior to hatching, resulting in both systems becoming lateralized due to the asymmetric light exposure.[12] In both species, keeping the eggs in the dark (and thus symmetrically exposing both eyes to no light) during the last days of incubation prevents the formation of stronger interhemispheric projections from either eye, and thus prevents the lateralization of the visual system.[12,20,24,25]

With the ascending visual pathways of the pigeon thus privileging the brain's left hemisphere with more complete visual information than the right, the question remains: does one side of the visual system have access to a more complete visual representation of the environment than the other? These questions – the degree of information transfer which occurs, and the potential asymmetry of any such transfer – prefigured the research program that ultimately yielded this thesis.

Piratical Neuroanatomy: Eye Patches on Pigeons

The complete decussation of the avian optic nerves means that testing of the information available to one eye or lateralized visual pathway can be achieved with the relatively simple procedure of temporarily covering one eye, allowing the bird to gather information with the other eye, then covering the originally

uncovered eye, and testing with the newly uncovered, "naïve" eye. If no integration is present, when tested with the second eye, the bird should display no familiarity with the stimuli or tasks experienced only with the first eye, mirroring the "two brains in one head" character demonstrated in human epilepsy patients treated by severing the *corpus callosum* (callosotomy).[26,27]

This effect has been demonstrated across numerous tasks in a variety of birds. In simple discrimination tasks, both pigeons and chickens have been shown to have difficulty identifying stimuli learned with one eye when viewing with the other,[18] and pigeons that successfully learn multi-layered transitive inference tasks show evidence of lack of interhemispheric transfer of that information when trained with one eye at a time on different transitive relationships.[25] Chickens fail to transfer pattern discrimination in monocular tasks,[28] but can overcome this failure when reinforced with food.[17]

This hemispheric independence presents a dual problem when thinking more deeply about the behavioural ecology of birds. Not only are birds reliant on vision to a degree greater than most mammals, but their characteristic mode of movement, flight, involves the very rapid acquisition and deployment of visual information.[29] Thus, their spatially oriented behaviour is visually demanding, and may be dramatically affected by their divided visual system. The spatial effects of hemispheric independence have been demonstrated in marsh tits - these birds cache food and recover it later, employing spatial memory to revisit their caches. Marsh tits caching food with one eye covered, which can accurately locate their caches with the eye exposed during caching, are not able to relocate their caches with the naïve eye when allowed to do so within three hours of caching.[30] However, the lateralization of visual memory storage manifests in this behaviour as well – left-eye-gathered caching sites apparently become available contralaterally

sometime between three and twenty-four hours following the caching, suggesting that in at least one direction, the limited brain integration has allowed some delayed transfer of the spatial memory for those sites.[31]

Navigation: A Long Term Visual Recognition Task

Navigation – very broadly, movement to some specific destination, whether at a long or short distance – is a demanding spatial task commonly undertaken by birds. In temperate regions, migration is common amongst bird species, with many species making yearly trips many thousands of kilometres long between wintering and summering grounds. On a more local scale, many birds have a reliable range within which they must navigate on a daily basis, and they must form visuo-spatial memories of these ranges to successfully navigate within them.

Homing is, in turn, a particularly high-fidelity form of navigation. The domesticated rock dove (*Columba livia*), otherwise known as the pigeon, is notable for its homing behaviour. Homing pigeons show a remarkable specificity of navigation in two dimensions.[32] First, their goal when homing is a very small destination: their loft. This is a small pigeon house of several square metres, rather than a broader general area, and a homing pigeon released from vast distances will reliably re-enter their loft on return. More remarkable still, however, is the reliability of the route flown in returning to the loft from a given release site. A pigeon released several times from the same site will, over the course of the first several flights, converge onto a route that, though idiosyncratic to each bird (and so not prefigured by the landscape itself), is tightly recapitulated on subsequent releases.[33] By affixing GPS loggers to pigeons engaged in homing, this route fidelity

has been demonstrated up to release distances of twenty-five kilometres from the loft, and it is likely that homing from distances still further will also demonstrate this route fidelity.[34]

These reliable homing routes are the result of visual interaction with the environment, further informed by other sensory modalities.[32] Pigeons have a suite of senses with which to gather the information necessary to home, including olfaction,[35,36] vision, and magnetoreception.[37-39] On initial release from a novel site, it is likely that their homing is guided through a combination of an olfactory "map", representing the relative scentscape of the birds' home environs,[40,41] and a "sun compass", based on movement and the position of the sun relative to its expected location at the home loft.[42] A prevalent current hypothesis is that whilst endeavouring this first return from a new location, the bird gathers information in the form of visual landmarks composed of prominent landscape features, including those with a linear character, such as rivers and roads.[32] On subsequent flights, these visual landmarks are consolidated into a chain of waypoints guiding the bird home on subsequent flights. After the first few flights (and generally from roughly the eighth flight onward) this results in near identical GPS traces of routes flown.[33] Thus, whilst homing relies on a variety of senses in its first instance, subsequent flights are a visual endeavour, as confirmed by the successful homing of pigeons which, having gained initial experience homing from a site, are subjected to olfactory anesthetization on subsequent flights.[43]

This complicated visual behaviour comprises two memory tasks: the recognition of the visual landmarks themselves, as well as the reproduction of the algorithmic relationship between them – the sequence of landmarks (so that the next can be identified as the pigeon passes each waypoint), and the distances and bearings flown to connect them. The current understanding of the avian brain, and the pigeon

brain in particular, suggests that the independence of the two halves of the visual system must affect these memory tasks. Chapter 2 of this thesis deals directly with the effects of the divided visual system on this memory task. Therein I establish that a pigeon, having established a recapitulated route whilst homing with only one eye (the other occluded), does not precisely recapitulate that route when homing only with the naïve eye, but does not revert entirely to the behaviour of a naïve pigeon, implying some degree of interhemispheric transfer of route information.

A Time Sensitive Phenomenon

The work in pigeons in Chapter 2 investigates the long-term integration of neurologically separated visual information, but does not and cannot inform our understanding of the instantaneous availability of new information to each brain hemisphere. Training a pigeon to home takes, at the very minimum, several days, and, as evinced by the aforementioned work in marsh tits,[30,31] some integration begins to occur within three to twenty-four hours of information acquisition.[44] Indeed, this time frame creates a general problem for investigating the hemispheric availability of purely visual information. Though the marsh tits could create caches with one eye and recover them with the other after three hours, this is a complex task including visual cues and spatial awareness, and could include a degree of dead reckoning via remembering the movements undertaken between sequentially stored caches. Meanwhile, most methods of training a bird on a visual stimulus – variations on rewarded operant conditioning, whether with keys, screens, or physical objects – require reinforcement training, causing the training period alone (as with pigeon homing) to extend well beyond even, in the longer estimation, a twenty-four hour delayed commencement of interhemispheric transfer of

information – to say nothing of the testing. Consequently, any long-trained visual identification behaviour is unsuitable to test the early or instantaneous availability of information, as the tests with the naïve eye, such as they occur any more than three hours later than the beginning of training, will be confounded by information transfer.

Pre-transfer information availability is fairly deemed irrelevant to homing or caching or reinforcement learning, when a three hour delay may pose no disadvantage, as the behaviour requiring access to the learned information is undertaken long thereafter anyway. However, the degree of integration instantaneous (or roughly such) to acquisition bears heavily on flight, conspecific and predator identification, and myriad other behaviours demanding rapid to immediate response to new visual information. Establishing a firm understanding of the strictures that avian neuroanatomy imposes on imminent reaction requires an answer to the experimental confounds of delay, hence my search for a study system where this could be avoided.

The solution was filial imprinting. This rapid form of learning occurs during juvenility, with a sensitive period beginning from under a day after hatching and lasting up to week in a precocial species,[45-47] and allows a newly hatched bird to quickly learn to identify its mother. This is a crucial adaptation, as the young are dependent on maternal proximity for their survival.[47] A young bird can become imprinted on a stimulus in as little as fifteen minutes and will show a preference for that stimulus, typically in the form of a following response.[48] The substrate of imprinting may be a visual or auditory stimulus, and in natural conditions, usually consists of both: the visual appearance of the mother and her auditory call.[46,47,49-52] Precocial imprinting, whereby the preference is developed almost immediately after hatching and manifests as faithful following of the mother, is characteristic of

Galloanserae, the fowl, and it is in the commonest of waterfowl (*Anseriformes*), the domestic Mallard (*Anas platyrhynchos domesticus*), that I investigated this phenomenon.

Because imprinting occurs so rapidly, a preference can be established by imprinting the subject and then testing it using the following response; multiple sessions of testing can thus occur within a matter of hours after initial learning. This made filial imprinting the ideal paradigm in which to test the timing of interhemispheric availability of information: or in other words, the ability of one eye to recognize a stimulus seen by the other, before the establishment of interhemispheric memory thereof by the commissures. Though imprinting is both visual and auditory in nature, it occurs when substrates are solely visual (i.e. when the training and testing chambers are kept silent),[47,52] and the target may be any moving stimulus. By contrasting stimuli identical in all respects but for colour, the substrate of imprinting preference may be reduced to a purely visual disparity – the preference for one colour over another. A duckling can be imprinted onto one coloured stimulus with one eye, then tested with the other eye for binary preference between the imprinted stimulus and an identical one of another colour. The imprinting is rapid and completed within a half hour, and the test can occur immediately thereafter to assess the immediate contralateral availability of the imprint. Tests can then be repeated over several hours to probe for the earliest evidence of transfer.

This question has an obvious follow-on. Namely, before the beginning of transfer, whenever it might begin, the bird is, theoretically, possessing different information in each hemisphere. Consequently, if an imprint can be formed by the left eye and isolated to the right hemisphere such that the left eye manifests the following effect but the right eye does not, it would follow that until the transfer begins to occur,

the right eye could imprint with fidelity on the opposite stimulus – resulting in a situation whereby one eye is imprinted on one stimulus and the other on a different one, with following responses dictated by which eye happens to be viewing its preferred target. Chapter 3 investigates these hypotheses and establishes that instantaneous transfer, though insufficient to allow maternal imprints to be immediately available to the contralateral eye, occurs sufficiently to avoid the potential harm that could be caused by a multiple imprint situation as described above.

From Brains to Bodies

Considerations of the laterality of pigeons' visual neurobiology recalled another laterality that revealed itself to be an information problem – and a visual one at that. The New Caledonian Crow (*Corvus moneduloides*) is an extensively studied species, the bird's natural use of sticks and leaf clippings as tools for foraging[53-55] having captivated researchers probing the flexibility of its problem solving[56] and comparing it to primates[57] and other species. The crows can successfully solve meta-tool problems[58] and creatively reshape tools into hooks when necessary,[59] and this behavioural flexibility has been invoked as an indication of the birds' intelligence.[60]

One aspect of their tool using behaviour that has been described is behavioural laterality. When manipulating short tools, the crows hold them in the beak, aligned to the beak's axis, by one end, and probe at the target site with the other. But when manipulating longer sticks, they clasp the tool in the beak at an angle along one side of the head, the proximate length protruding back along the side and behind the head, and the working end protruding forward at an angle, toward the side contralateral to the held segment of the tool.[61] Each New

Caledonian Crow shows a reliable side preference when holding tools this way – the birds are either reliable "right-holders" or "left-holders" – while the species has thus far exhibited no overall bias, with roughly equal numbers of right- and left-holders tested.[62]

This lateral preference is superficially similar to handedness exhibited by humans, other primates, and in some birds, whereby one hand (or foot) is preferentially used in tasks of fine manipulation.[63-65] However, unlike handedness, defined as the preference for one bilaterally symmetric limb over its counterpart, the crows' tool-holding preference involves only one extremity, the head and neck. Holding the tool to one side or another does not alter which extremity will manipulate the tool, rendering the preference for one side over the other a completely different problem. One hypothesis as to the origin of this laterality is that this is learned, acquired from observing parents while learning to use tools. A tempting conclusion, this idea cannot wholly explain the phenomenon, because tool use in the species occurs in juveniles never exposed to tool use by either conspecifics or humans exhibiting both tool use itself and laterality thereof.[66]

The most likely candidate, then, for the origin of this laterality of behaviour was some laterality of anatomy or physiology – something concretely beneficial in holding tools to one side or the other. One such corporeal laterality relevant to the sides of the head is eye dominance. This lateral preference involves the superiority of one eye in focusing through narrow apertures, through which binocular viewing of a target is not possible.[64,65] Well known to archers, marksmen, billiards players, and astronomers, the preferential use of the dominant eye to peer through a narrow hole or down a thin shaft increases the acuity and accuracy of aim and manipulation of whatever is being viewed.[65]

In humans and primates, eye dominance does not correlate with handedness, and at first approach there would seem little reason to suspect that this avian handedness analogue – "beak-sidedness" – would not similarly lack correlation. However, a bird manipulating a tool in its beak suffers an anatomical limitation that an ape manipulating a tool in its hands does not. A left handed archer with a dominant right eye may raise the bow to loosing position with either hand, and tilt and turn his head as necessary to peer down the arrow shaft with either eye – and the same is true of an ape threading a branch into a termite colony or a microscopist manoeuvring the slide stage with either hand. The crows, limited as they are to holding tools in their beaks and thus limited in what portion of the tool each eye can see,[7] may display a laterality of tool holding in order to manipulate a tool to their visual preference. Here again, information management plays a critical role in determining behaviour, which may be manipulated to maximise accuracy of incoming visual information.

Chapter 4 investigates the origin of the crows' tool-holding laterality in this context. I hypothesized that tool use laterality could in fact be a function of and manifestation of eye laterality. To explore this hypothesis I investigated the correlation between these preferences. Furthermore, the dictation of tool use laterality by eye laterality suggests that this too is an information problem – a bird attempting to finely manoeuvre a tool must require a high degree of accuracy of information regarding the relative positions of the tool tip and target, and the effects of each movement thereupon. Chapter 4 suggests that maintaining access to this visual information drives the laterality of tool use in New Caledonian Crows.

So What is Information?

Having thus far dwelt at length on the topic of information – how it is gathered and stored and the lengths to which animals may go to obtain it accurately – the subject of the nature of information itself has been left vague. Quite deliberately so, because it is an open, controversial question – difficult to test and difficult even to coherently characterize.[67] Chapter 5 thus deals with how animals internalize information, how it is represented, and what is being created in the brain when we claim that "information has been acquired." It is not itself directly related to interocular transfer, but is, however profoundly relevant to the greater issue of what we mean when addressing questions of information acquisition, storage and transfer.

To understand why this is the case, let us consider what may happen when an animal perceives a stimulus. Suppose the animal is a bird, and so observes the stimulus primarily through visual sensation. Even supposing it is something relatively simple – for instance a matte, red sphere about ten centimetres in diameter – there are countless qualities that, without which, any description of the stimulus must also include a number of similar objects, through generalization. An exact representation of the sphere constructed from the activation of a circular zone of the retina must include its precise shade and luminance, its inferred three-dimensional shape (no small matter – this alone includes several sub-characteristics: the shadowing around it, the implied curvature of the facing surface, the inference that the opposite surface may be similarly curved – these and many other subtle characteristics must be correct to prevent false positives from matte, red ovoids at a fortuitous angle), its size relative to the visual field and the observer's viewing distance, its matte finish and the precise degree of gloss it exhibits. If any of these or other qualities are not precisely identified, attempts to

discriminate the observed stimulus from all others will inevitably yield false positives.[68]

No animal behaviour (save perhaps humans' own exactitude in some art or engineering) requires this discrimination be perfect, and most tasks likely involve a vastly more general capability of recognition – predator, prey, and conspecific identification indeed require that the discrimination being undertaken allows for generalization: a zebra that avoids only one specific lion, with mane colouration just so, will quickly find itself consumed by a slightly varied lion. Meanwhile, a bird will need a much more specific description of its mate in order to successfully distinguish him from roughly similar conspecifics – though not so specific that he is ignored when standing at an unusual angle to the viewer. This sort of discrimination is a signal detection problem like any other; in the case of the lion and zebra, a "miss", could be fatal, while for mate identification, a "false alarm" results in wasted time and resources. An animal's representational ability needs to have a repertoire of sensitivity levels to avoid Type 1 errors (false positives) when pursuing some goal, and Type 2 errors (false negatives) when avoiding some peril. Identifying a potential mate, or a homing landmark, or potential tool requires recognizing that a presented stimulus is an exemplar from a category – whether broadly (e.g. mates) or narrowly (e.g. my mate) defined.

These categories are perceptual concepts – abstractions (to a great or small degree) that define an in-group and out-group, and are not coterminous with any specific stimulus.[69] Even in highly specific cases (my mate), the category may include multiple visual stimuli – a mallard, in the water, facing me, conveys very different visual information to the brain compared with the same mallard, standing on grass, facing away. These perceptual concepts are one of the ways some animals represent information gathered. The in-groups and out-groups overlap with each

other; categories and subcategories encompass the characteristics of incoming information, contextualizing and structuring it in useful form.[70]

In humans, this seems intuitive – much ink has been spilled discussing the ubiquity of categorization, and this is usually attributed to the value of this sort of pattern recognition to survival.[71] It is then quite reasonable to suspect that, at the very least, some other species might do something similar. And indeed, several apes and a few birds have been shown capable of establishing perceptual concepts and categorizing stimuli accurately therein.[69,72,73] In a classic example, pigeons were able to learn to categorize images based on whether or not they contained a human, trees, or bodies of water, and did so accurately regardless of the use of novel images, variance in the angle at which the subject was photographed, and other manipulations.[74] The category employed, "pictures containing humans" (or trees, or water), is quite irrelevant to the ordinary discrimination behaviour of a pigeon, and so it was necessary to extensively train the pigeons through operant conditioning, both to acquire the perceptual concept in question and also to apply it in the form of a discriminating peck. However, this is no reason to suspect that this capability for concept formation is not in regular employ in a pigeon's normal behaviour, when the adaptive relevance of a category is higher, for example "things which are food". It is the arbitrariness of the experimental concept that both allows for controlled testing and mandates rewarded training. Empirically observed perceptual concept formation generally occurs under an associative learning protocol.

Relational concepts are still more abstract. Whilst perceptual concepts concern the qualities of a stimulus itself and the categories they place it within, relational concepts compare two or more qualities of stimuli, and are quite intangible to and independent of the stimuli themselves.[70] One of the most

straightforward relational concepts is whether two (or more) stimuli are equal or different.[75] This is a distinction which may have degrees: *ceteris paribus,* an ovoid and a sphere are in some sense more similar than a sphere and a cube, and when more than two stimuli are compared, one iteration may have more similar elements than others (for example, fifteen identical objects and one different one versus sixteen different elements);[76,77] but it is also one with a bright line – true sameness, by most definitions, brooks no variation, even if some differences are more pronounced than others. Regardless of the specifics of definition, sameness and difference differ from perceptual concepts in that they are further abstracted from the stimulus itself, and indeed are quite divorced from any of its qualities: a yellow object is inexorably part of the perceptual category "yellow", but its membership in "same" or "different" is entirely dependent on context.

Relational concepts, being much more abstract, have proven more difficult for animals to learn in operant trials – and indeed many studies claiming to show relational concept learning fail to adequately do so. One method, which has been employed to claim relational concept learning for the honeybee,[78] is to train an animal that a reward may be obtained by discriminating, from between two stimuli, the one that matches a previously presented sample (for example, presenting for discrimination a red and blue circle, having previously presented a blue circle as the sample, and offering reward when the blue circle is selected, then transferring to a new modality, such as stimuli differing in shape but equal in colour). In the case of the bees, some were trained to always choose the "same" stimulus, others the "different" stimulus, and then successfully discriminated in a different modality in their transfer trials. This is then taken as evidence for relational concepts in the honeybee.[78] However, this is not robust evidence of relational concept learning, but rather, a case of cued discrimination masked by the relation between stimuli

and the cue itself. The rule "if blue, pick blue" is not substantially different from the rule "if tone one sounds, pick blue". The transfer to other modalities is more convincing, but does not suggest that the bees are learning a concept of "sameness" and "difference", but rather have acquired the operant rules of "match to sample" or "non-match to sample"; the learned rule does not abstract from the qualities of the stimuli, it relies upon them.

Relational matching to sample is a stronger test of relational concept formation, and in this task, only some primates[77,79-82] and a few birds: parrots,[83] corvids,[84,85] and, under extensive training, pigeons,[86-88] have demonstrated the abstraction in question.[89] Here, the cue presented is itself composed of two stimuli that manifest sameness or difference (two red spheres, or a red sphere and a red cube, for example). The animal must then discriminate between two further such pairings, one same and one different, and select the pair exhibiting the same relationship as the sample, even though all of the testing stimuli are novel. This is a more difficult abstraction, and all species that have thus far successfully demonstrated this ability have done so after extensive, rewarded reinforcement.[85] One species of crow has spontaneously demonstrated this ability, the first non-primate to do so, but then only when relational trials were interspersed between long-term, conditioned identity matching trials, in the context of rewarded discrimination.[85]

Concepts Are Not a Luxury

The rarity and difficulty of training animals to demonstrate the use of concepts, especially the more abstract relational concepts, might at first suggest that such abstraction is only possible for highly intelligent species: primates and the most cognitively developed of birds. This is, however, an unlikely conclusion – as

has already been discussed, any meaningful use of information must involve contextualizing it conceptually. Rather than concepts being cognitively beyond most animals, it is possible that it is instead the causal nature of operant conditioning and other reinforcement protocols that provides the stumbling block, as concept formation seems necessary to a much wider range of animals than primates and the most intelligent birds. It seemed reasonable, then, to investigate whether this phenomenon could be demonstrated in an unexpected species by removing it from the reinforcement-learning context; still testing concept learning, but avoiding the potential cognitive difficulty of operant conditioning.

To investigate this, I returned to filial imprinting in ducks to subject concept learning outside the operant context to a "hard" test. The popular notion of filial imprinting is that it is automatic and limited in scope: a bird sees its mother, an imprint is formed, and this "visual snapshot" is reflexively followed until maturation. Not only is this description inaccurate[90-92] (as there is ample evidence of young fowl forming a second imprint when their original imprinting substrate is removed),[93,94] but any imprint based solely on a visual snapshot is doomed to yield potentially disastrous false negatives. A mother duck with extended wings, partially submerged when floating, standing partway behind a tree, or holding its head and neck in a previously unseen position will differ substantially from any given memory "snapshot" upon which such a theoretical imprint is based. A duckling operating on a single snapshot (or even an array of several) could fail to recognize its own mother when presented in some context not previously seen during the sensitive period for imprinting. The mechanism must therefore be more flexible, and young birds have indeed been shown capable of imprinting on more abstract substrates, such as biological motion patterns,[92] and under limited, specific circumstances, small numerical values.[91]

To create a "hard" test for non-reinforced concept learning, in Chapter 5 I tested whether day-old ducklings could acquire the relational concepts of sameness and difference through imprinting, using moving pairs of stimuli as surrogate mothers, and testing with novel stimuli entirely distinct from the imprinted pairs, but exhibiting or not exhibiting the same relational concept. This is a "hard" test on two fronts: since imprinting is a highly specific form of identification – having only one true positive – the need for abstraction in normal maternal imprinting is less than in a broader identification task. Concept learning through imprinting is thus particularly suggestive of a general ability for concept formation, since imprinting is among the less conceptual tasks an animal might face. Secondly, this test employs the significantly more abstract and difficult problem of relational concept learning. The work in Chapter 5 does not use an operant conditioning protocol: the ducklings receive no material reward at any time, and no extensive training. Samples are presented once, and then discrimination is tested – the behaviours are spontaneous, because the context is that of a biologically prepared propensity.

Chapter 5 represents the portion of this thesis that involved the greatest risk, deliberately posing a hard test for a potentially absent ability. It was undertaken despite this because of its profound bearing on the nature of information acquired and deployed by animals, a matter of major relevance to the problem of inter-ocular information transfer discussed in Chapters 2, 3, & 4. The results of Chapter 5 suggest that concept learning, and in particular relational concept learning, occur spontaneously in ducklings, and that this ability may be necessary for the survival of many species that have not thus far evidenced this capability.

In Summary

This thesis is about how birds manage visual information flow from beginning to end: the adaptations to ensure accurate information; the neuroanatomical strictures placed on storing that information; and the cognitive capabilities evolved to make that information useful to a bird engaged in complex tasks of identification in its natural context. The results herein, especially Chapters 2, 3, & 4, contribute to our understanding of how a large brain built on non-mammalian architecture deals with the visually demanding umwelt of living like a bird. By studying the possibilities opened by this neuroanatomy, the limitations it imposes, and the adaptive works-around evolved to combat these limitations, we can better understand the evolutionary pressures that have shaped our own brains, and continue to shape the behaviour of other vertebrates. Chapter 5 proposes that the psychological architecture of concept formation and abstraction, often attributed only to the most intelligent species, may be much more widespread, and indeed may be necessarily so: adaptively crucial to flexible behaviour. Evolutionary success for birds means responding correctly to a constantly varying, and informationally rich environment. This thesis explores how this is done.

References

1 Koch, K. *et al.* How much the eye tells the brain. *Current Biology* **16**, 1428-1434 (2006).

2 Heesy, C. P. & Hall, M. I. The nocturnal bottleneck and the evolution of mammalian vision. *Brain, behavior and evolution* **75**, 195-203 (2010).

3 Martin, G. R. What is binocular vision for? A birds' eye view. *Journal of Vision* **9**, 14 (2009).

4 Fox, R., Lehmkuhle, S. W. & Bush, R. C. Stereopsis in the falcon. *Science* **197**, 79-81 (1977).

5 Howard, I. P. & Rogers, B. J. *Perceiving in Depth, Volume 2: Stereoscopic Vision*. 561-562 (Oxford University Press, 2012).

6 McFadden, S. A. in *Perception and Motor Control in Birds* 54-73 (Springer, 1994).

7 Troscianko, J., von Bayern, A. M. P., Chappell, J., Rutz, C. & Martin, G. R. Extreme binocular vision and a straight bill facilitate tool use in New Caledonian crows. *Nature communications* **3**, 1110 (2012).

8 Rogers, L. J. Development and function of lateralization in the avian brain. *Brain research bulletin* **76**, 235-244 (2008).

9 Rogers, L. J., Vallortigara, G. & Andrew, R. J. *Divided brains: the biology and behaviour of brain asymmetries*. (Cambridge University Press, 2013).

10 van der Knaap, L. J. & van der Ham, I. J. M. How does the corpus callosum mediate interhemispheric transfer? A review. *Behavioural brain research* **223**, 211-221 (2011).

11 Watanabe, S. Interhemispheric transfer of visual discrimination in pigeons with supraoptic decussation (DSO) lesions before and after monocular learning. *Behavioural brain research* **17**, 163-170 (1985).

12 Ströckens, F., Freund, N., Manns, M., Ocklenburg, S. & Güntürkün, O. Visual asymmetries and the ascending thalamofugal pathway in pigeons. *Brain Structure and Function* **218**, 1197-1209 (2013).

13 Skiba, M., Diekamp, B., Prior, H. & Güntürkün, O. Lateralized interhemispheric transfer of color cues: evidence for dynamic coding principles of visual lateralization in pigeons. *Brain and language* **73**, 254-273 (2000).

14 von Fersen, L. & Güntürkün, O. Visual memory lateralization in pigeons. *Neuropsychologia* **28**, 1-7 (1990).

15 Pecchia, T., Gagliardo, A., Filannino, C., Ioalè, P. & Vallortigara, G. in *Behavioral Lateralization in Vertebrates* 107-124 (Springer, 2013).

16 Voneida, T. J. & Mello, N. K. Interhemispheric projections of the optic tectum in pigeon. *Brain, behavior and evolution* **11**, 91-108 (1975).

17 Gaston, K. E. Interocular transfer of pattern discrimination learning in chicks. *Brain research* **310**, 213-221 (1984).

18 Diekamp, B., Prior, H. & Güntürkün, O. Functional lateralization, interhemispheric transfer and position bias in serial reversal learning in pigeons (Columba livia). *Animal Cognition* **2**, 187-196 (1999).

19 Parsons, C. H. & Rogers, L. J. Role of the tectal and posterior commissures in lateralization of the avian brain. *Behavioural brain research* **54**, 153-164 (1993).

20 Manns, M. & Ströckens, F. Functional and structural comparison of visual lateralization in birds–similar but still different. *Frontiers in psychology* **5** (2014).

21 Deng, C. & Rogers, L. J. Prehatching visual experience and lateralization in the visual Wulst of the chick. *Behavioural brain research* **134**, 375-385 (2002).

22 Skiba, M., Diekamp, B. & Güntürkün, O. Embryonic light stimulation induces different asymmetries in visuoperceptual and visuomotor pathways of pigeons. *Behavioural brain research* **134**, 149-156 (2002).

23 Manns, M. & Güntürkün, O. Dual coding of visual asymmetries in the pigeon brain: the interaction of bottom-up and top-down systems. *Experimental brain research* **199**, 323-332 (2009).
24 Güntürkün, O., Stüttgen, M. C. & Manns, M. Pigeons as a model species for cognitive neuroscience. *e-Neuroforum*, 1-7 (2014).
25 Manns, M. & Römling, J. The impact of asymmetrical light input on cerebral hemispheric specialization and interhemispheric cooperation. *Nature communications* **3**, 696 (2012).
26 Corballis, M. C. Can commissurotomized subjects compare digits between the visual fields? *Neuropsychologia* **32**, 1475-1486 (1994).
27 Asadi-Pooya, A. A., Sharan, A., Nei, M. & Sperling, M. R. Corpus callosotomy. *Epilepsy & Behavior* **13**, 271-278 (2008).
28 Gaston, K. E. Lack of interocular transfer of pattern discrimination learning in chicks. *Brain research* **171**, 339-343 (1979).
29 Gunturkun, O. in *Sturkie's Avian Physiology* (ed G. Causey Whittow) (Academic Press, 1999).
30 Sherry, D. F., Krebs, J. R. & Cowie, R. J. Memory for the location of stored food in marsh tits. *Animal Behaviour* **29**, 1260-1266 (1981).
31 Clayton, N. Lateralization and unilateral transfer of spatial memory in marsh tits. *Journal of Comparative Physiology A* **171**, 799-806 (1993).
32 Biro, D., Meade, J. & Guilford, T. Familiar route loyalty implies visual pilotage in the homing pigeon. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 17440-17443 (2004).
33 Meade, J., Biro, D. & Guilford, T. Homing pigeons develop local route stereotypy. *Proceedings of the Royal Society B: Biological Sciences* **272**, 17 (2005).
34 Biro, D., Meade, J. & Guilford, T. Route recapitulation and route loyalty in homing pigeons: pilotage from 25 km? *Journal of Navigation* **59**, 43-53 (2006).
35 Gagliardo, A. Forty years of olfactory navigation in birds. *The Journal of experimental biology* **216**, 2165-2171 (2013).
36 Gagliardo, A. *et al.* Olfactory lateralization in homing pigeons: a GPS study on birds released with unilateral olfactory inputs. *The Journal of experimental biology* **214**, 593-598 (2011).
37 Wiltschko, W. & Wiltschko, R. Magnetic orientation and magnetoreception in birds and other animals. *Journal of Comparative Physiology A* **191**, 675-693 (2005).
38 Keeton, W. T. Magnets interfere with pigeon homing. *Proceedings of the National Academy of Sciences* **68**, 102-106 (1971).
39 Wallraff, H. G. & Foà, A. The roles of olfaction and magnetism in pigeon homing. *Naturwissenschaften* **69**, 504-505 (1982).
40 Papi, F., Fiore, L., Fiaschi, V. & Benvenuti, S. Olfaction and homing in pigeons. *Monitore Zoologico Italiano-Italian Journal of Zoology* **6**, 85-95 (1972).
41 Wallraff, H. G. Pigeon homing from unfamiliar areas: an alternative to olfactory navigation is not in sight. *Communicative & integrative biology* **7**, 6929-6943 (2014).
42 Guilford, T. & Taylor, G. K. The sun compass revisited. *Animal behaviour* **97**, 135-143 (2014).
43 Hartwick, R. F., Foa, A. & Papi, F. The effect of olfactory deprivation by nasal tubes upon homing behavior in pigeons. *Behavioral Ecology and Sociobiology* **2**, 81-89 (1977).
44 Clayton, N. S. & Krebs, J. R. Lateralization and unilateral transfer of spatial memory in marsh tits: are two eyes better than one? *Journal of Comparative Physiology A* **174**, 769-773 (1994).
45 Lorenz, K. Imprinting. *Auk* **54**, 245-273 (1937).

46 Klopfer, P. H. & Gottlieb, G. Imprinting and behavioral polymorphism: Auditory and visual imprinting in domestic ducks (Anas platyrhynchos) and the involvement of the critical period. *Journal of comparative and physiological psychology* **55**, 126 (1962).
47 Bateson, P. P. G. The characteristics and context of imprinting. *Biological Reviews* **41**, 177-217 (1966).
48 Bateson, P. & Jaeckel, J. B. Chicks' preferences for familiar and novel conspicuous objects after different periods of exposure. *Animal Behaviour* **24**, 386-390 (1976).
49 Miller, D. B. & Gottlieb, G. Maternal vocalizations of mallard ducks (Anas platyrhynchos). *Animal Behaviour* **26**, 1178-1194 (1978).
50 Fischer, G. J. Auditory stimuli imprinting. *journal of Comparative and Physiological Psychology* **61**, 271 (1966).
51 Dyer, A. B. & Gottlieb, G. Auditory basis of maternal attachment in ducklings (Anas platyrhynchos) under simulated naturalistic imprinting conditions. *Journal of Comparative Psychology* **104**, 190 (1990).
52 Boyd, H. & Fabricius, E. Observations on the incidence of following of visual and auditory stimuli in naive mallard ducklings (Anas platyrhynchos). *Behaviour* **25**, 1-14 (1965).
53 Bluff, L. A., Troscianko, J., Weir, A. A. S., Kacelnik, A. & Rutz, C. Tool use by wild New Caledonian crows Corvus moneduloides at natural foraging sites. *Proceedings of the Royal Society B: Biological Sciences* **277**, 1377-1385 (2010).
54 Chappell, J. & Kacelnik, A. Tool selectivity in a non-primate, the New Caledonian crow (Corvus moneduloides). *Animal cognition* **5**, 71-78 (2002).
55 Hunt, G. R. Manufacture and use of hook-tools by New Caledonian crows. *Nature* **379**, 249-251 (1996).
56 Taylor, A. H., Elliffe, D., Hunt, G. R. & Gray, R. D. Complex cognition and behavioural innovation in New Caledonian crows. *Proceedings of the Royal Society B: Biological Sciences* **277**, 2637-2643 (2010).
57 McGrew, W. C. Is primate tool use special? Chimpanzee and New Caledonian crow compared. *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**, 20120422 (2013).
58 Taylor, A. H., Hunt, G. R., Holzhaider, J. C. & Gray, R. D. Spontaneous metatool use by New Caledonian crows. *Current Biology* **17**, 1504-1507 (2007).
59 Weir, A. A. S., Chappell, J. & Kacelnik, A. Shaping of hooks in New Caledonian crows. *Science* **297**, 981-981 (2002).
60 Emery, N. J. & Clayton, N. S. The mentality of crows: convergent evolution of intelligence in corvids and apes. *science* **306**, 1903-1907 (2004).
61 Weir, A. A. S., Kenward, B., Chappell, J. & Kacelnik, A. Lateralization of tool use in New Caledonian crows (Corvus moneduloides). *Proceedings of the Royal Society of London. Series B: Biological Sciences* **271**, S344-S346 (2004).
62 Rutledge, R. & Hunt, G. R. Lateralized tool use in wild New Caledonian crows. *Animal Behaviour* **67**, 327-332 (2004).
63 Izawa, E.-I., Kusayama, T. & Watanabe, S. Foot-use laterality in the Japanese jungle crow (Corvus macrorhynchos). *Behavioural processes* **69**, 357-362 (2005).
64 Bourassa, D. Handedness and eye-dominance: A meta-analysis of their relationship. *Laterality: Asymmetries of Body, Brain and Cognition* **1**, 5-34 (1996).
65 Merrell, D. J. Dominance of eye and hand. *Human Biology* (1957).
66 Kenward, B., Weir, A. A. S., Rutz, C. & Kacelnik, A. Behavioural ecology: Tool manufacture by naive juvenile crows. *Nature* **433**, 121-121 (2005).
67 Dall, S. R. X., Giraldeau, L.-A., Olsson, O., McNamara, J. M. & Stephens, D. W. Information and its use by animals in evolutionary ecology. *Trends in ecology & evolution* **20**, 187-193 (2005).

68 Treisman, A. Feature binding, attention and object perception. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **353**, 1295-1306 (1998).
69 Zentall, T. R., Galizio, M. & Critchfied, T. S. Categorization, concept learning, and behavior analysis: an introduction. *Journal of the experimental analysis of behavior* **78**, 237 (2002).
70 Zentall, T. R., Wasserman, E. A., Lazareva, O. F., Thompson, R. K. & Rattermann, M. J. Concept learning in animals. *Comp Cogn Behav Rev* **3**, 13-45 (2008).
71 Simon, H. A. Invariants of human behavior. *Annual review of psychology* **41**, 1-20 (1990).
72 Chittka, L. & Jensen, K. Animal cognition: concepts from apes to bees. *Current Biology* **21**, R116-R119 (2011).
73 Lazareva, O. F. & Wasserman, E. A. Category learning and concept learning in birds. *The making of human concepts*, 151-172 (2010).
74 Herrnstein, R. J., Loveland, D. H. & Cable, C. Natural concepts in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes* **2**, 285 (1976).
75 Wasserman, E. A. & Young, M. E. Same–different discrimination: The keel and backbone of thought and reasoning. *Journal of Experimental Psychology: Animal Behavior Processes* **36**, 3 (2010).
76 Fagot, J., Wasserman, E. A. & Young, M. E. Discriminating the relation between relations: the role of entropy in abstract conceptualization by baboons (Papio papio) and humans (Homo sapiens). *Journal of Experimental Psychology: Animal Behavior Processes* **27**, 316 (2001).
77 Wasserman, E. A., Fagot, J. & Young, M. E. Same–different conceptualization by baboons (Papio papio): The role of entropy. *Journal of Comparative Psychology* **115**, 42 (2001).
78 Giurfa, M., Zhang, S., Jenett, A., Menzel, R. & Srinivasan, M. V. The concepts of 'sameness' and 'difference'in an insect. *Nature* **410**, 930-933 (2001).
79 Katz, J. S., Wright, A. A. & Bachevalier, J. Mechanisms of same-different abstract-concept learning by rhesus monkeys (Macaca mulatta). *Journal of Experimental Psychology: Animal Behavior Processes* **28**, 358 (2002).
80 Truppa, V., Piano Mortari, E., Garofoli, D., Privitera, S. & Visalberghi, E. Same/different concept learning by capuchin monkeys in matching-to-sample tasks. *PloS one* **6**, e23809 (2011).
81 Wright, A. A., Santiago, H. C. & Sands, S. F. Monkey memory: Same/different concept learning, serial probe acquisition, and probe delay effects. *Journal of Experimental Psychology: Animal Behavior Processes* **10**, 513 (1984).
82 Wright, A. A., Shyan, M. R. & Jitsumori, M. Auditorysame/different concept learning by monkeys. *Animal Learning & Behavior* **18**, 287-294 (1990).
83 Pepperberg, I. M. Acquisition of the same/different concept by an African Grey parrot (Psittacus erithacus): Learning with respect to categories of color, shape, and material. *Animal Learning & Behavior* **15**, 423-432 (1987).
84 Wilson, B., Mackintosh, N. J. & Boakes, R. A. Transfer of relational rules in matching and oddity learning by pigeons and corvids. *The Quarterly Journal of Experimental Psychology* **37**, 313-332 (1985).
85 Smirnova, A., Zorina, Z., Obozova, T. & Wasserman, E. Crows spontaneously exhibit analogical reasoning. *Current Biology* **25**, 256-260 (2015).
86 Cook, R. G., Kelly, D. M. & Katz, J. S. Successive two-item same-different discrimination and concept learning by pigeons. *Behavioural Processes* **62**, 125-144 (2003).
87 Katz, J. S. & Wright, A. A. Same/different abstract-concept learning by pigeons. *Journal of Experimental Psychology: Animal Behavior Processes* **32**, 80 (2006).

88 Zentall, T. R. & Hogan, E. Same/different concept learning in the pigeon: the effect of negative instances and prior adaptation to transfer stimuli. *Journal of the Experimental Analysis of Behavior* **30**, 177-186 (1978).
89 Wright, A. A. & Katz, J. S. Mechanisms of same/different concept learning in primates and avians. *Behavioural Processes* **72**, 234-254 (2006).
90 Wood, J. N. Characterizing the information content of a newly hatched chick's first visual object representation. *Developmental science* **18**, 194-205 (2015).
91 Rugani, R., Regolin, L. & Vallortigara, G. Imprinted numbers: newborn chicks' sensitivity to number vs. continuous extent of objects they have been reared with. *Developmental science* **13**, 790-797 (2010).
92 Regolin, L., Tommasi, L. & Vallortigara, G. Visual perception of biological motion in newly hatched chicks as revealed by an imprinting procedure. *Animal Cognition* **3**, 53-60 (2000).
93 Bolhuis, J. J. & Bateson, P. The importance of being first: a primacy effect in filial imprinting. *Animal Behaviour* **40**, 472-483 (1990).
94 Bateson, P. & Horn, G. Imprinting and recognition memory: a neural net model. *Animal Behaviour* **48**, 695-715 (1994).

Chapter 2

Asymmetric Visual Input and Route Recapitulation in Homing Pigeons

Antone Martinho III, Dora Biro, Tim Guilford, Anna Gagliardo, & Alex Kacelnik

Originally published October 7, 2015, Volume 282.1816, Proceedings of the Royal Society B. Published typesetting included in Appendix.

"Statistics are the triumph of the quantitative method, and the quantitative method is the victory of sterility and death."

Hilaire Belloc

Abstract

Pigeons (*Columba livia)* display reliable homing behaviour, but their homing routes from familiar release points are individually idiosyncratic and tightly recapitulated, suggesting that learning plays a role in route establishment. In light of the fact that routes are learned, and that both ascending and descending visual pathways share visual inputs from each eye asymmetrically to the brain hemispheres, we investigated how information from each eye contributes to route establishment, and how information input is shared between left and right neural systems. Using onboard GPS loggers, we tested 12 pigeons' route fidelity when switching from learning a route with one eye to homing with the other, and back, in an A-B-A design. Two groups of birds, trained first with the left or first with the right eye, formed new idiosyncratic routes after switching eyes, but those that flew first with the left eye formed these routes nearer to their original routes. This confirms that vision plays a major role in homing from familiar sites and exposes a behavioural consequence of neuroanatomical asymmetry whose ontogeny is better understood than its functional significance.

Introduction

Birds and mammals moving in familiar landscapes acquire, process, store, and use visual information, but whilst in the mammalian brain bilateral projections from the optic chiasma and inter-hemispheric connectivity through the corpus callosum ensure that visual information is readily available bilaterally, in birds visual pathways are almost completely decussated at the optic chiasm, and there is no corpus callosum. These differences make integration of visual input from the two eyes very different between these two vertebrate classes, and lead to differences in learning and memory.[1] Pigeons using visual landmarks to fly homewards from familiar locations[2] provide an opportunity to study how visual information acquired by each eye is stored and made available to contralateral visual control of navigation.

How pigeons find their way home has been subject of extensive research and lively debate for many years.[3,4] Field experiments have shown that pigeons compute their homeward bearing based on a 'map' (information on the bird's position relative to the loft, most likely derived from olfactory cues when in unfamiliar territory)[2,3,5] and use a time-sensitive sun compass[6,7] or a magnetic compass[8] in assuming a homeward bearing and navigating to their loft. As they acquire experience with an area, pigeons progressively incorporate visual cues in the form of landmarks to engage in pilotage – or navigation by a series of visual landmarks – with reliance on compass information decreasing with experience.[9-12] On-board global positioning system (GPS) loggers have greatly enriched the study of pigeon homing, revealing that pigeons released repeatedly from the same site form individually idiosyncratic routes that are tightly recapitulated on subsequent flights.[10,11,13] Furthermore, their routes often incorporate long linear landmarks such as roads, railway lines, and rivers.[9]

The pigeon visual system is lateralized both at neuroanatomical and at the functional level, as shown in birds tested in laboratory cognitive tasks.[14] Functional

asymmetries have also been found for spatial tasks involving memorization and processing of cues in both laboratory settings and natural behaviours, such as homing.[15] In particular, the functional contribution of the left and right side of the brain in homing behaviour has been the subject of experiments focussing on the hippocampal formation and olfactory and visual systems.[15,16] Although both the left and right parts of the hippocampal formation seem to be involved in familiar landmark-based navigation,[17] an intact left hippocampus is needed for olfactory map learning in young pigeons raised confined,[18] likely due to the critical role of the left hippocampus in sun compass mediated spatial learning[19] through which the association of wind born odours with wind direction is memorised. As concerning the olfactory system, an asymmetric involvement in favour of the left piriform cortex and of the right nostril has been highlighted.[15]

Within the visual system, pigeons' optic nerves completely decussate at the optic chiasm, projecting exclusively to the tectum contralateral to the input eye,[20] meaning that direct visual inputs to a hemisphere can be eliminated by capping an eye. The ascending tectofugal and thalamofugal visual pathways do allow for indirect visual input to the contralateral hemisphere,[21] but while each tectum projects to the ipsilateral diencephalic nucleus rotundus roughly equally, more projections from the right tectum are shared contralaterally to the left nucleus rotundus and entopallium than vice versa.[1,14,22] This asymmetry is developmentally related to the orientation of the pigeon embryo prior to hatching, whereby the right eye is exposed to more light during incubation.[14,23] The importance of this light exposure to interhemispheric integration has been shown in visual transitive inference tasks performed by monocularly occluded pigeons.[20] It has been suggested that memory formation and storage may be lateralised, with predominance of the left hemisphere,[24] and further asymmetries of projections have been found in both the ascending and descending visual systems.[25]

This fits into the wider model of the avian visual system as being lateralised differently for specific tasks and roles.[26] Nonetheless, this model is largely derived from chickens, and it is likely that detailed patterns of lateralisation differ between pigeons, chickens, and other birds.

Here, we investigate whether the asymmetries of the visual memory system are reflected in pigeons' route fidelity when switching from monocular homing with an 'experienced' to a 'naïve' eye. We trained twelve pigeons to fly home under reversible, non-invasive monocular occlusion (Fig. 1) for 18 flights before subsequently flying another 18 flights from the same release point with the opposite eye blocked. This was followed by five flights with the original eye blocked, and finally by five binocular flights, completing a total of 46 flights per bird, in four phases. Our results reflect a poverty of interhemispheric exchange of visual homing information and a hemispheric imbalance of information availability in the pigeon brain.

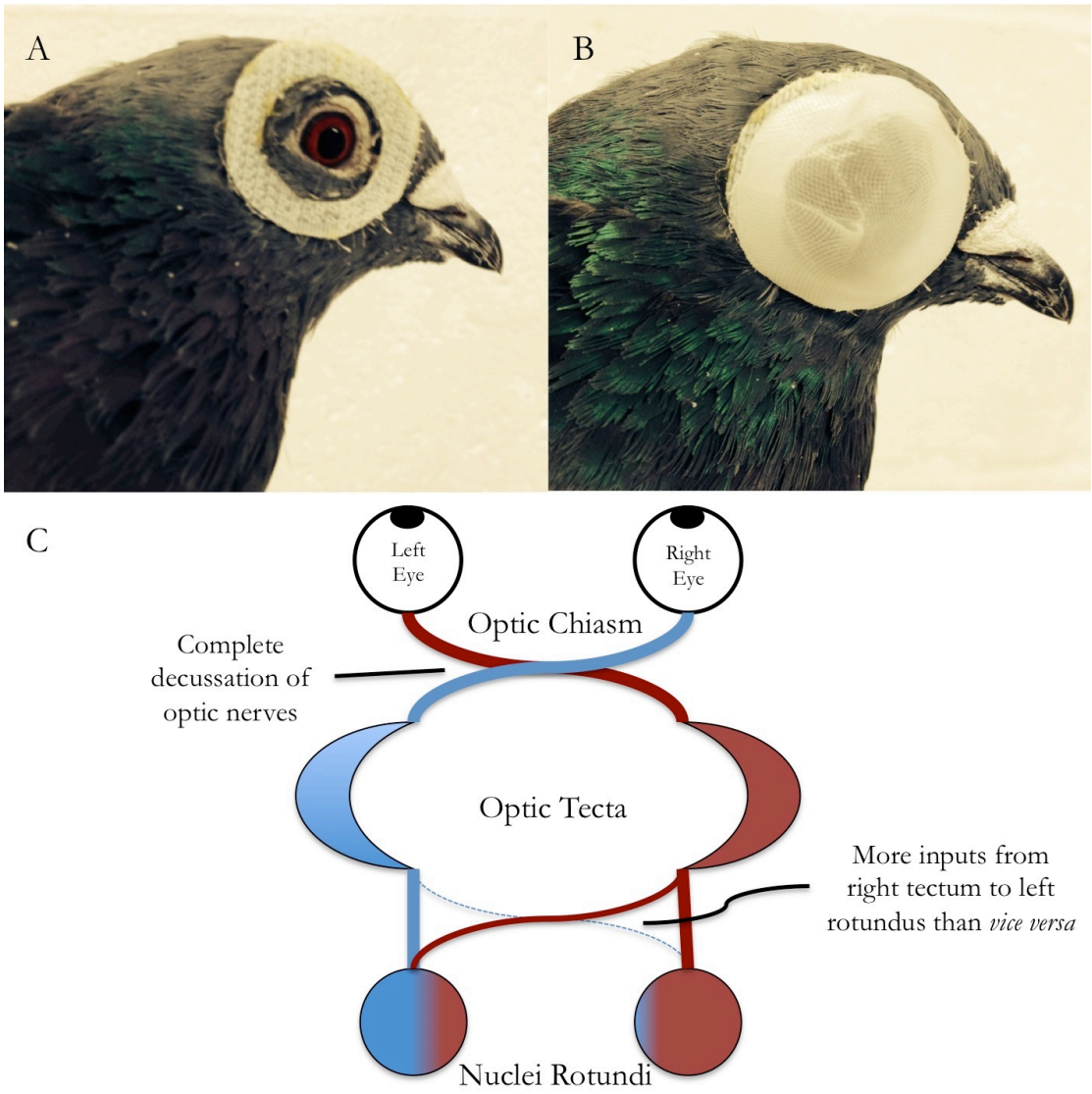


Figure 1. Pigeon with eye ring and eye cap and generalized structure of pigeon tectofugal system

A) Each pigeon was fitted with two eye rings as shown. The 'hook' side of Velcro was used for the ring attached to the pigeons' feathers as its thinner profile ensured forward vision would not be compromised. B) The eye caps were constructed of a double layer of flexible etched plastic. The seam in the eye cap was consistently oriented posteriorly to ensure a homogenous image in the forward dimension. C) Schematic of the pigeon tecto-fugal visual system, detailing the asymmetry of inputs. Diagram adapted from [23].

Experimental Procedures

Subjects & Materials

Sixteen homing pigeons two years of age and of both sexes were used. Pigeons were housed at the Oxford University Field Station, Wytham, UK, where they had also been bred. Pigeons were kept at free feeding weight with unlimited access to water, grit, and mixed grain food, and allowed to fly freely from the loft daily.

Twelve experimental birds were prepared for attachment of GPS logging devices by trimming non-flight feathers on their backs and applying a 4cm strip of Velcro with fast drying glue. All GPS devices were initially attached via the Velcro method, but in cases in which the strip became loose during the course of the experiment, the attachment method was switched to the use of an elasticated harness (for further details, see 'Irregularities', below). Rings of Velcro to facilitate eye cap attachment were glued around each eye as in Figure 1. QStarz BT-Q1300ST 5Hz personal GPS loggers were modified for attachment to pigeons, by removing the outer plastic covering. Eye caps (see Figure 1) consisted of a 2 cm diameter disk composed of a double layer of etched plastic to allow for light penetration but prevent shape identification. The remaining four birds formed a control group and were prepared in the same fashion for GPS attachment, but were not fitted with eye rings.

All pigeons received basic training of at least three flock releases and three solo releases from locations roughly aligned with the four cardinal directions, approximately 2 km from the loft, alternated to prevent directional bias. All training flights were flown binocularly.

Releases

Releases investigated homing in birds completely naïve to the release site. Pigeons were transported to the release site in individual chambers within two pigeon

boxes, which allowed thorough airflow but prevented the pigeons seeing out of the boxes. The boxes were transported by automobile, with all windows lowered to allow the pigeons access to the olfactory scentscape during travel. Upon arrival at the release site, the boxes were removed from the car and the birds were allowed 7 minutes before the first release. During this time, the pigeons were fitted with their eye caps over the appropriate eye without removal from the box and GPS loggers were prepared. All birds' experimental releases were solo flights and birds were released 7 minutes apart to prevent ad hoc flock formation. The pigeons had no previous homing experience beyond basic training. A single release point was used, but all analyses compared changes in route within subject and between flights, rather than location of route with reference to the landscape. Any bias toward landmarks from this release site is therefore unlikely to have affected our results. Furthermore, the use of a single release site minimised loss of birds.

Experimental Groups

The 12 experimental birds were divided into two groups, one of seven (LRLB group) and one of five (RLRB group). The groups initially contained eight birds each, but one bird from the LRLB group and three birds from the RLRB group failed to home during the first flight (See Losses and Irregularities, below).

The pigeons were released from a novel location 3.8 km from the loft. The releases comprised 50 flights in four phases over the course of three months.

Phase 1 (Flights 1–18) consisted of first-eye training and testing. The Left-Right-Left-Binocular (LRLB) group flew with their left eye (right eye covered), and the Right-Left-Right-Binocular (RLRB) group with the right eye (left eye covered) during all flights in Phase 1. Twenty releases were performed, and the first 18 of the 20 Phase 1flights completed by each bird were included in analysis. The final two flights were

reserves, included to accommodate occasions when a bird missed a flight for one of a number of reasons or a GPS logger failed (see Irregularities, below). In cases in which a bird did not miss two flights within the set of 20, any flights beyond the 18th (1-2 flights) were excluded from consideration.

Phase 2 (Flights 19–36) consisted of second-eye training and testing, and followed the same pattern as Phase 1. In Phase 2, the LRLB group flew with their right eye (left covered), and the RLRB group with their left eye (right covered). As in Phase 1, 20 releases were performed, and the first 18 successfully flown by each pigeon were included in analysis.

Phase 3 (Flights 37–41) consisted of re-testing the first eye. The LRLB group flew with their left eye and the RLRB group with their right eye. All pigeons flew five flights in Phase 3.

Phase 4 (Flights 42–46) consisted of binocular testing. All 12 pigeons flew 5 flights with both eyes uncovered.

Control Group

The four control birds were released binocularly 18 times from the same site as the experimental birds, following the same protocols. The first 13 flights comprised training and the last five flights (Flights 14-18) were used to determine 'normal high-familiarity homing' for comparison to experimental birds. The control birds did not experience any irregularities, and the results from the controls were highly consistent, allowing the use of only four animals, in keeping with the goal of reduction of animals in research.

Data Processing

GPS traces of flight paths were converted into comma-separated values format by proprietary software included with the GPS loggers. All subsequent processing and analysis of flight paths was performed in MATLAB. All flights were trimmed for speed (retaining all points for which speed was greater than 0.5m/s for 30 seconds before or after) to exclude waiting time before release and after re-entering the loft. Flights were then trimmed to exclude points within 200 meters of the start and endpoints to exclude time when all flights are constrained to be very close together. To assist comparisons with other experiments, a table of commonly used pigeon homing metrics before the advent of GPS location may be found in Electronic Supplementary Materials.

Nearest Neighbour Analysis

The Nearest Neighbour analysis holds one of the two flights compared in reference, and for each point along the reference measures the distance to the closest point on the compared flight in any direction (see Figure 5A, inset). For each pairwise comparison, both flights are held in reference and compared to the other in turn, and the mean of these two series of distances provides the mean nearest-neighbour distance between the two flights.

Losses and Irregularities

Losses

The first flight of the experimental releases was performed with a slightly modified version of the eye cap that incorporated a layer of tracing paper to partially occlude light. Four of 16 birds failed to home during this first flight. As a result of this high failure rate, the eye-caps were restructured to exclude the tracing paper and

include a second layer of etched plastic, preserving the shape disruption but allowing for still more light penetration. Following this modification, the remaining 12 birds homed successfully. Among the 12 birds that successfully homed during the first flight, performance was reduced, with long routes very far from the beeline. However, only the final ten flights in each phase are considered included in the analysis, so any reduced performance in the first flight is not reflected in comparison of the first phase to the other phases.

Irregularities

On some occasions, a bird did not produce data during a given flight. When this occurred, a reserve flight was used. All such irregularities occurred during Phases 1 and 2, and in no case did a bird require more than two reserve flights. Causes of irregularities included sudden weather changes, GPS device failure, and birds not-yet-returned from free flight around the loft. Additionally, nine birds' Velcro strips fell off during the course of the experiment. As this was a result of feather moulting beneath the strip, the strip could not be reattached until the following season, and so was replaced by a 'backpack' consisting of a fabric pouch to hold the GPS logger held to the pigeon by elasticated straps that go around the wings and across the keel. In seven of these cases, the bird in question was mounted with the backpack and excluded from flights for the remainder of the day to allow it to become accustomed to the backpack. Each of these birds wore the backpack for the remaining duration of the experiment and during all remaining flights.

Results

The twelve experimental birds flew in two groups defined by the sequence of which eye was available (*i.e.* the eye which was not occluded) in each phase of experimentation: 'left-right-left-binocular' (hereafter LRLB) and 'right-left-right-binocular' (hereafter RLRB). A control group of four birds flew 18 non-occluded flights. Positional fixes recorded at 5Hz by onboard GPS loggers allowed us to reconstruct the pigeons' flights (see Methods), and to compare paths flown. The set of the first 18 flights for each group (excluding the two provisional flights) is referred to as 'Phase 1' and the second 18 flights (again, excluding the provisional flights, and numbered from 19 in keeping with the same exclusion in Phase 1), flown with the other eye, as 'Phase 2'. The subsequent five flights, flown with the original eye comprise 'Phase 3'. For clarity, except where otherwise indicated, the descriptors 'left-eye' and 'right-eye' refer to the eye that is open (not occluded) for the flight being described.

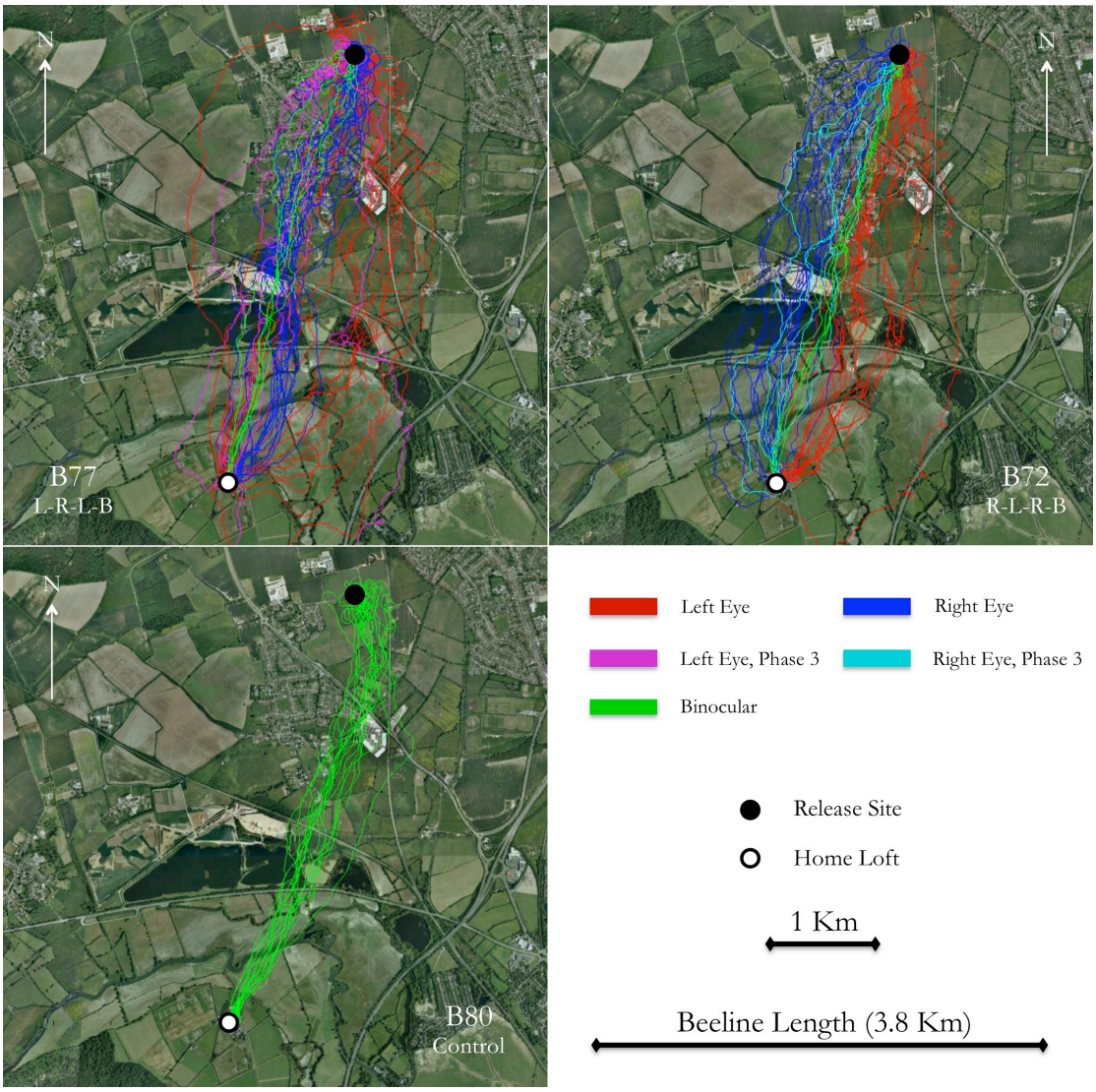


Figure 2. Examples of flight series for two experimental and one control pigeon

Each experimental pigeon flew 46 flights in either a '18 left, 18 right, 5 left, 5 binocular' or '18 right, 18 left, 5 right, 5 binocular' pattern (referring to the eye open, not the eye occluded). Bird IDs and treatment received are indicated in a bottom corner of each panel. Left-eyed flights are shown in red (magenta for Phase 3, bird B77 only), while right-eyed flights are blue (cyan for Phase 3, bird B72 only). Binocular flights are shown in green. The control pigeons flew 18 flights each, all of which were binocular.

Visual inspection of the trajectories revealed consistent patterns, as displayed by exemplars in Figure 2. For all experimental birds, the first several flights show properties distinct from subsequent monocular flights and from all control flights. In these early monocular flights, the pigeons' routes deviate from the beeline between the release site and the loft in the direction of the available eye. As the pigeons become more experienced with a given eye, the routes migrate toward the beeline. Furthermore, during early flights, the pigeons often perform many tight loops around the open eye – clockwise when the right eye is open and counter-clockwise when the left eye is open, possibly attempting to gather information from a 360° field of view, a behaviour not previously described, as far we are aware. This behaviour may have been undertaken to keep the visual field of the open eye fixed, or moving slowly, as the open eye facing toward the centre of the loop would reduce the visual flow to that eye – possibly facilitating focus on the birds' surroundings such that visual landmarks for subsequent flights could form. At no point in the study was a bird seen to loop around an occluded eye. Control birds did perform loops, but as both eyes were open, flights included loops in both directions. We quantified each bird's looping rate as loops per kilometre of route flown, excluding loops made around the release site and home loft (Fig. 3). Among experimental birds, looping rate begins high and subsides through subsequent flights, before returning to a high rate when the occluded eye is switched, and subsiding in a similar pattern. We compared the looping rate across flights within each of the controls, and the first two phases of each of the LRLB and RLRB birds, using a Two-Way Repeated Measures ANOVA. The 18-flight phases of the experimental birds were considered separately, such that the ANOVA included 5 groups of 18 repeated measures (flights). The rate of looping decreases significantly across flights ($F(17,340) = 7.162$, $p < 0.001$), indicating that reduced looping rates correlate with increased experience homing over a given route. There was no

significant interaction between looping rate and group, suggesting that this effect is similar across groups. Both experimental groups showed an increase in looping rate following the first eye switch, presumably due to poor or incomplete transfer, or poor contralateral input of visual information. The subsiding of loop rate throughout each phase suggests that loops may provide information during early flights and thus become unnecessary as the birds become familiar with their recapitulated route. It is also possible that, rather than having a homing information role, high looping rate reflects general acclimatization to a new monocular condition, as it is seen each time a naïve eye is flown uncovered for the first time.

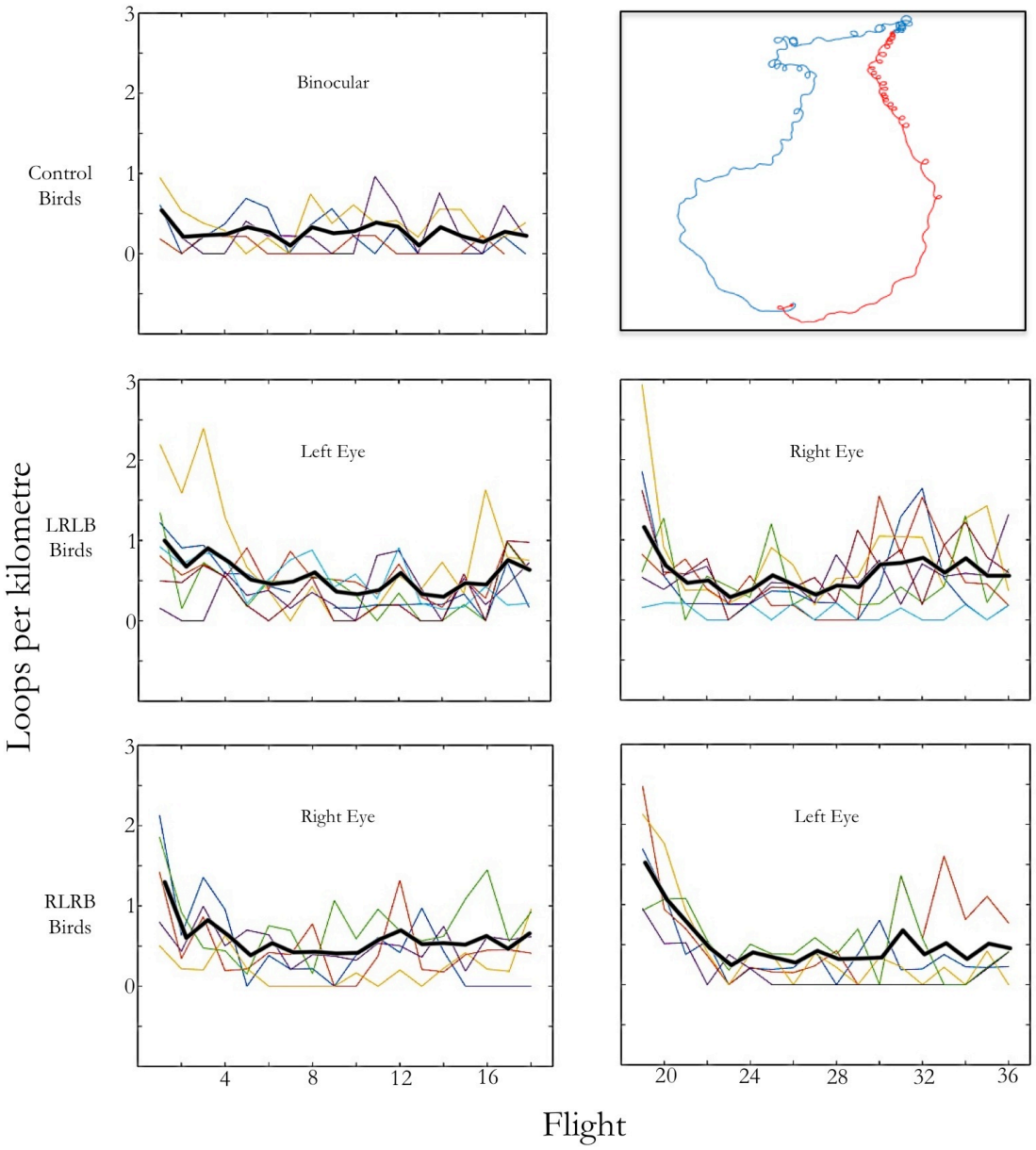


Figure 3. Looping Rate Analysis

Monocularly occluded birds perform tight loops (approximately 25-50m in diameter) around the open eye during early flights as seen in the inset, top right (the blue track is a right-eyed bird, the red, left). The binocular controls and both phases of experimental flights show significant decrease in looping rate throughout the 18 flights. The heavy black lines show the mean value across pigeons in the group for each flight. High early looping rates may reflect information gathering by visually hemicompromised pigeons.

To test whether information input to the contralateral hemisphere was more effective in one direction than the other, we assessed how the birds' trajectories changed across phase changes. To do this, we used a distance-to-beeline analysis (Figure 4). Each flight was sampled at points representing GPS positional fixes collected at 5Hz. This analysis computes the mean perpendicular distance in metres between the beeline and each point in a given flight, assigning positive values to scores left of the beeline and negative values to those to the right, with respect to the direction of flight. Figure 4A shows the beeline analysis for each flight. To compare the degree to which routes changed across phases, we computed the difference in distance to beeline between each bird's first flight after an eye switch and the mean of the ten flights before the switch (Figure 4B), for both the initial switch from Phase 1 to 2 (*i.e.* switching to a 'naïve' eye) and the subsequent switch from Phase 2 to 3 (*i.e.* switching back to the eye that became 'experienced' in Phase 1). We calculated the magnitude of the change from pre-switch mean to the first post-switch flight for each bird, and compared the groups via two-sample t-tests, as shown in Figure 4B. The average (± SE) signed magnitude of the changes in distance to the beeline after the first (Naïve) switch was -250.2±67.0m for the LRLB group and 506.9±98.5m for the RLRB group. In addition to the obvious sign difference (Figure 4B), the absolute value of the difference between the first and second phase for the RLRB group was significantly greater than for the LRLB group ($p = 0.049$, two sample t-test), corresponding with the move across the beeline seen in the RLRB graph, indicating that the asymmetric effect of the treatment was true at individual level and not an artifact of averaging. This implies that the LRLB group's route was less affected by the eye switch than was the case for the RLRB group. It is possible that information concerning a general spatial affinity for the landmarks acquired with the left eye/right hemisphere system (LRLB group) reached the left hemisphere via an indirect input to

a greater degree than in the opposite direction (RLRB group). There was no significant difference between groups in the absolute value of change between the second and third phases ($p = 0.580$).

While the LRLB birds did fly nearer to their Phase 1 routes in Phase 2 than was the case for RLRB birds, the beeline analysis does not determine the fidelity of route recapitulation (similar mean distance to the beeline can result from different trajectories). For this reason, route fidelity was examined using a 'nearest neighbour analysis' (Fig. 5A, inset), in which each flight within a bird's series was compared to the immediately preceding flight. The details of this analysis are presented in 'Methods'. The nearest neighbour analysis shows how the route differs between consecutive pairs of flights,[27] providing a measure of variability or fidelity of a bird's recapitulation of its route. Figure 5A shows the nearest neighbour results for the controls and the first phases of the two experimental groups. The controls show a rapid progression from early variability to asymptotic fidelity. These results were compared to the first phase of both experimental groups via Two-Way Repeated Measures ANOVAs. All three groups showed a significant main effect of decrease in nearest neighbour distance with experience across the 17 pairwise comparisons ($F(16, 176) = 1.827$, $p = 0.031$), but no significant difference between the groups ($F(32, 176) = 0.876$, $p = 0.662$). The Phase 2 results of the experimental groups where compared in the same way, with both groups again showing a significant decrease across the 18 pairwise comparisons (including the cross-phase comparison of the last flight in Phase 1 to the first in Phase 2), ($F(17, 170) = 7.098$, $p < 0.001$), and a significant difference in nearest neighbour values between the two groups ($F(17, 170) = 2.167$, $p = 0.007$). This is consistent with the results of the beeline analysis, which showed that the RLRB group displayed a larger shift in spatial location of route across the eye-switch. Both groups were affected by the switch from the first eye to the second – the high nearest neighbour values imply that the first

flight of Phase 2 did not recapitulate the last flight of Phase 1 in either group. This is consistent with the findings of the beeline analysis, which showed that both groups' routes changed, albeit to different degrees, across the initial eye switch. This implies that whilst both groups achieved consistent recapitulation by the end of each of Phases 1 and 2, both groups also modified their routes after the switch, as nearest neighbour distances increased at the beginning of Phase 2.

The high nearest neighbour values at the beginning of Phase 2 do not exclude the possibility that the birds produced high variability as a result of the eye-switch, but then settled back to the same recapitulated route as before the switch – especially the LRLB birds, which were shown in the beeline analysis to achieve similar scores across phases. However, as we noted earlier, similarity of beeline score does not imply route recapitulation, and a visual inspection of the final five flights for each bird in each of Phases 1 and 2 reveals that this was not the case. Figure 5B shows representative examples of these flights for each group (equivalent figures for all birds are supplied in Figure S1). As can be seen, while within phase flights are near to each other, routes differ across phases. Some birds, especially in the LRLB group, did fly very near their previous route, but the shape of their route differed between the two Phases. This suggests that while the LRLB birds did show slightly higher route fidelity after the eye switch than the RLRB birds, the complete information necessary for precise recapitulation, perhaps including sequence, bearings, or other onward guidance connecting one landmark to another, was not sufficiently available to either group to allow strict recapitulation.

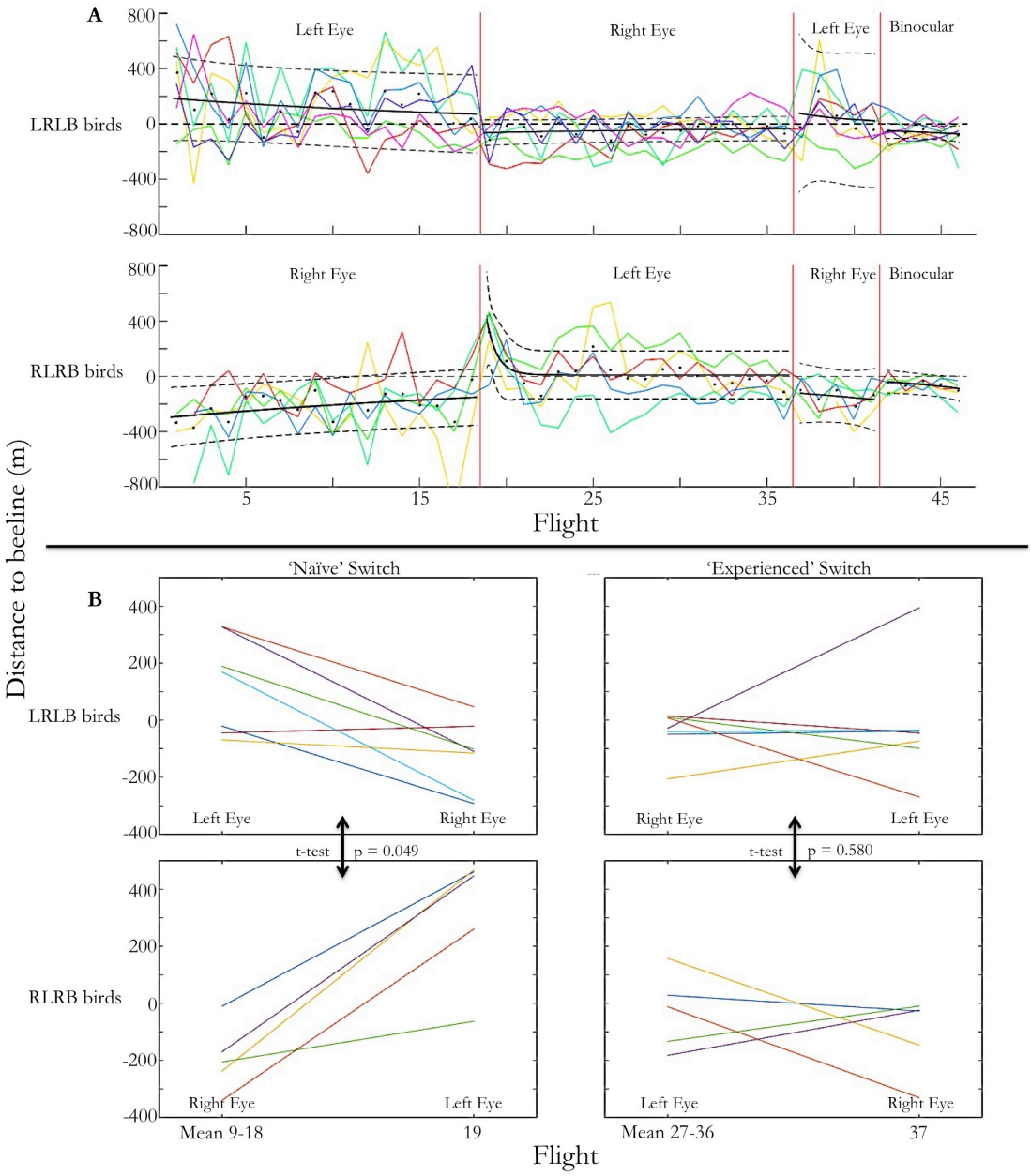


Figure 4. Beeline Analysis

The beeline analysis calculates the distance to the beeline for each point along a flight, and gives the mean of these distances as the score for that flight. A) Each bird's score is shown for each flight. The black lines show a fitted exponential curve with 95% confidence intervals (fitted parameters are given in Electronic Supplementary Materials). The 'left eye' and 'right eye' labels refer to the open eye. Points to the right of the beeline, with reference to the direction of motion, are scored as negative values, while those to the left are positive. B) For each of the two eye-switches from Phase 1 to 2 and Phase 2 to 3, the mean of the final ten flights before the switch is compared to the first flight after the switch.

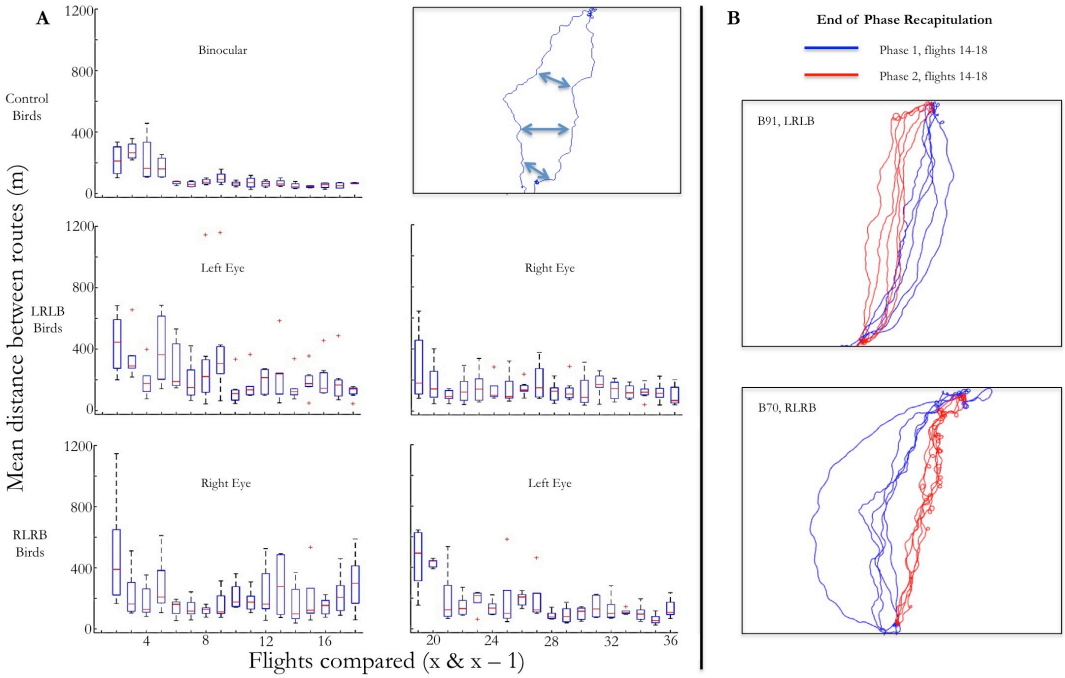


Figure 5. Nearest neighbour analysis of route development

A) The nearest neighbour analysis calculates the degree to which a flight has changed with respect to the previous flight, as seen in the inset, top right (see Methods for details). The blue arrows represent the shortest distances between the two flights, computed at each point in the flight (here, three are shown for clarity). The pairwise comparisons of flights in each group (Controls, LRLB, and RLRB) are shown as boxplots about the median. The 'left eye' and 'right eye' labels refer to the open eye. (Note that since there is no flight previous to the first, the first frame is left empty.) Recapitulation occurs when nearest neighbour values remain low, while adjacent high values indicate variability of route. B) Examples showing that both LRLB and RLRB birds recapitulated over a different route in each phase.

Discussion

Our results indicate that in pigeons input of visual homing information from each eye is not closely integrated with input from the other eye, that the level of integration is asymmetric, and that this asymmetry has functional consequences. Pigeons' routes learned solely with one eye are not recapitulated when flying with the other, naïve eye, and this failure to recapitulate occurs regardless of which eye learns first, though birds that home first monocularly with the left eye fly significantly nearer to their left-eyed route when subsequently flying right-eyed than vice versa. This suggests that the various commissures in the pigeon visual pathway allow insufficient contralateral input of route information for strict recapitulation using the naïve eye. However, route recapitulation by visual landmarks requires both visual and non-visual memory, as a pigeon must both recognize each landmark visually and remember which direction to take and which landmark comes next in sequence at each point to follow its established route tightly. It is thus possible that though this strict sequence was lost in both groups, the LRLB group's more general visual recognition and attraction to the previously learned landmarks may have endured across phases, and may reflect the asymmetry of the pigeon's visual pathway, especially in the ascending tecto-fugal pathway.

Concordant with the asymmetry present in the commissures of the tecto-fugal visual pathway, birds flying with the right eye after learning initially with the left eye, as seen in the LRLB group, fly significantly nearer to their original (left-eye), first-learned route than birds flying in the opposite order. Thus, although neither group using the second eye followed the precise set and sequence of visual landmarks learned with the first eye, the asymmetry of the pigeon visual system[1,14,23] means that information acquired with the left eye is more readily available to control visually-guided behaviour by the alternative eye than the opposite. Even after repeated experience, the Phase 2

routes of the RLRB group settle farther from their initial (left eyed acquired) routes than those of the LRLB birds, possibly because the weaker projections from the left optic tectum to the right nucleus rotundus are much reduced relative to the opposite side,[1,14,23] though other neural asymmetries may contribute to this difference.[25] Thus, though neither eye can access the exact sequence of landmarks determining the route established with the other eye, the information acquired with the left eye is more readily available to both eyes than that acquired with the right,[23] allowing birds flying right-eyed after left to fly more closely to the routes learned with the left eye. The fact that no significant change in route was found in either group upon returning to the first eye in the third phase is consistent with previous observations that pigeons retain learned recapitulated routes over long periods of time. In both groups, by the beginning of the third phase, eighteen flights had been flown by each eye, thus allowing whichever eye was used in the third phase to access those visual memories and home accordingly.

Our beeline analysis results further suggest that the difference in performance cannot be solely attributed to lateralization – the superiority of one eye or hemisphere over the other for homing performance. If this were the case, we would expect to find similar performance for a given eye across both groups in all phases. Instead we see that homing performance for a given eye varies depending on the sequence of training and previous experience. This suggests that our results derive from the asymmetry of visual inputs to the two hemispheres, rather than simple superiority of one hemisphere or eye.

These results confirm the suspected effects of neuroanatomical division and asymmetry of visual processing, and contribute to our understanding of visual lateralisation in homing for pigeons with some homing experience. A previous study on monocular homing inferred the importance of the eyes for homing lateralisation

from different homing speed and vanishing bearings with each eye, but argued that since both naïve and experienced birds showed vanishing bearing deflection and lateralization of performance, this was not an effect of visual memory but potentially of lateral specialization for magneto-reception or optic flow.[28] However, naïve and experienced pigeons navigate by different strategies; namely, naïve pigeons rely mostly on site-specific compass orientation, while experienced pigeons transition to pilotage-based orientation within the familiar area.[29] By utilizing GPS technology that allows investigation of the fidelity of homing to previously recapitulated routes, we have shown that the better performance of the LRLB pigeons in Phase 2 (when flying with the previously naïve right eye) is likely a result of the left hemisphere's more complete visual memory of homing routes. The Phase 2 results indicate that pigeons experienced with both eyes separately show right eye/left hemisphere superiority in homing, a pattern previously noted in pigeons flying monocularly after simultaneous binocular experience,[30] and in pigeons navigating in an indoor arena strewn with visual landmarks.[31]

Finally, our results reaffirm the importance of vision itself in the process of pigeon homing. At one time the subject of debate, the degree of relevance of visual information in allowing pigeons to reliably home remains in question. While the formation of, and loyalty to, individually idiosyncratic homing routes have been taken as key support for the importance of visual information and memory in familiar area homing,[9] these inferences were indirect. The current study is the first to assess route recapitulation whilst manipulating visual information input directly during homing, and demonstrates that monocular homing performance follows well the pattern established in other purely visual tasks. Pigeons undertaking a monocular binary discrimination task after binocular training show better performance with the right eye than with the left, again likely a consequence of the right eye's access to a more complete visual

memory.[32] Our results demonstrate a similar pattern for familiar area homing, and thus situate this homing within the pigeon's visually controlled behaviours.

Ethics

All experiments were conducted with the approval of the University of Oxford's Zoology Animal Welfare Ethical Review Board (Ref. APA/1/5/Zoo; date 7/11/2013).

Data Accessibility

GPS tracks of all flights in the experiment are available on Dryad, DOI: 10.5061/dryad.2c47d.

Competing Interests

We have no competing interests.

Author Contributions

The experiment was devised by AM with input and advice from DB, TG, AG, and AK. Birds were prepared for experiments by AM, with assistance from AK and DB. Pigeon releases were carried out by AM. Data processing was carried out by AM, and analysis was performed by AM with input and assistance from DB, TG, AG, and AK. AM wrote the manuscript with editing and assistance from DB, TG, AG, and AK.

Acknowledgements

We thank Isobel Watts, Lucy Taylor, and Takao Sasaki for their cooperation in the shared use of the pigeon colony, and Mateo Lewis and Emma Truswell for assistance with pigeon releases. We are grateful to Lucy Larkman and David Wilson for their technical support and help with animal husbandry. We thank Benjamin Pettit for instructing AM on the processing of pigeon flight data in MATLAB and Richard

Mann and Robin Freeman for writing much of the code used in this processing. DB was supported by a Royal Society University Fellowship.

Electronic Supplementary Material

Curve fitting details for Figure 4

All curves were fitted using the equation $y = ae^{bx}$.

Coefficients and goodness of fit:

LRLB Flights 1–18

Coefficients (with 95% confidence bounds):
a = 184.8 (25.49, 344.2)
b = -0.05244 (-0.1559, 0.05105)

Goodness of fit:
SSE: 2.271e+05
R-square: 0.06127
Adjusted R-square: 0.002601
RMSE: 119.1

LRLB Flights 19–36

Coefficients (with 95% confidence bounds):
a = -72.33 (-123.6, -21.11)
b = -0.03577 (-0.1135, 0.04197)

Goodness of fit:
SSE: 2.728e+04
R-square: 0.05989
Adjusted R-square: 0.001134
RMSE: 41.29

LRLB Flights 37–41

Coefficients (with 95% confidence bounds):
a = 98.05 (-652.5, 848.6)
b = -0.307 (-3.716, 3.102)

Goodness of fit:
SSE: 4.817e+04
R-square: 0.0742
Adjusted R-square: -0.2344
RMSE: 126.7

LRLB Flights 42–46

Coefficients (with 95% confidence bounds):

a = -49.34 (-89.03, -9.653)
b = 0.08863 (-0.1333, 0.3105)

Goodness of fit:
SSE: 598.7
R-square: 0.3341
Adjusted R-square: 0.1122
RMSE: 14.13

RLRB Flights 1–18

Coefficients (with 95% confidence bounds):
a = -302.6 (-412.4, -192.9)
b = -0.03731 (-0.07745, 0.002829)

Goodness of fit:
SSE: 1.235e+05
R-square: 0.189
Adjusted R-square: 0.1383
RMSE: 87.85

RLRB Flights 19–36

Coefficients (with 95% confidence bounds):
a = 1661 (-3127, 6448)
b = -1.649 (-4.376, 1.077)

Goodness of fit:
SSE: 1.114e+05
R-square: 0.4802
Adjusted R-square: 0.4477
RMSE: 83.46

RLRB Flights 37–41

Coefficients (with 95% confidence bounds):
a = -120.9 (-279.7, 38.02)
b = 0.07576 (-0.2912, 0.4427)

Goodness of fit:
SSE: 9118
R-square: 0.1366
Adjusted R-square: -0.1512
RMSE: 55.13

RLRB Flights 42–46

Coefficients (with 95% confidence bounds):
a = -41.37 (-92.54, 9.797)
b = 0.1723 (-0.1461, 0.4907)

Goodness of fit:
SSE: 1399
R-square: 0.4783
Adjusted R-square: 0.3044
RMSE: 21.59

Classical Pigeon Metrics

Efficiency scores

Route efficiency is calculated as the beeline distance from release point to home loft divided by the total distance travelled. Mean efficiency ± standard deviation is given for the selection of flights indicated.

	Phase 1, Flights 1-5	Phase 1, Flights 14-18	All Phase 1 Flights	Phase 2, Flights 1-5	Phase 2, Flights 14-18	All Phase 2 Flights	All Phase 3 Flights	All Phase 4 Flights
LRLB Birds	0.582 ± 0.15	0.711 ± 0.09	0.669 ± 0.12	0.711 ± 0.10	0.699 ± 0.11	0.709 ± 0.10	0.670 ± 0.14	0.798 ± 0.07
RLRB Birds	0.616 ± 0.15	0.705 ± 0.11	0.683 ± 0.13	0.642 ± 0.11	0.784 ± 0.09	0.729 ± 0.11	0.721 ± 0.10	0.792 ± 0.08
Controls	0.695 ± 0.20	0.759 ± 0.19	0.759 ± 0.16					

Homing time scores

Homing time is the time, in seconds, from release to landing at loft. Mean homing time ± standard deviation is given.

	Phase 1, Flights 1-5	Phase 1, Flights 14-18	All Phase 1 Flights	Phase 2, Flights 1-5	Phase 2, Flights 14-18	All Phase 2 Flights	All Phase 3 Flights	All Phase 4 Flights
LRLB Birds	641.2 ± 680.6	307.0 ± 57.1	473.2 ± 533.4	328.9 ± 61.5	352.1 ± 91.8	361.7 ± 267.2	475.6 ± 530.9	439.9 ± 663.1
RLRB Birds	510.4 ± 425.8	324.6 ± 84.0	440.0 ± 444.6	373.3 ± 109.1	306.1 ± 55.4	339.7 ± 77.3	379.3 ± 175.0	266.7 ± 77.5
Controls	251.1 ± 110.2	548.3 ± 359.4	329.8 ± 235.1					

Virtual vanishing bearing scores

Virtual vanishing bearing is the direction of flight, relative to the release point, when the bird first exceeds a distance of 2.5km from the release point. It is expressed in degrees, with 0 or 360 indicating North, 90 East, 180 South, and 270 West. Mean bearing ± standard deviation is given.

	Phase 1, Flights 1-5	Phase 1, Flights 14-18	All Phase 1 Flights	Phase 2, Flights 1-5	Phase 2, Flights 14-18	All Phase 2 Flights	All Phase 3 Flights	All Phase 4 Flights
LRLB Birds	185.8 ± 16.5	192.1 ± 9.1	190.3 ± 12.0	198.4 ± 7.7	196.3 ± 5.6	197.6 ± 6.3	193.7 ± 7.9	196.4 ± 3.7
RLRB Birds	225.4 ± 44.8	203.3 ± 12.2	209.4 ± 26.4	189.6 ± 11.8	196.6 ± 4.3	192.6 ± 8.7	201.1 ± 10.6	195.1 ± 3.4
Controls	196.2 ± 7.4	193.7 ± 3.8	194.7 ± 5.6					

Virtual vanishing time scores

Virtual vanishing time is the time in seconds between release and when the bird reaches the point at which virtual vanishing bearing (above) is taken. Mean vanishing time ± standard deviation is given.

	Phase 1, Flights 1-5	Phase 1, Flights 14-18	All Phase 1 Flights	Phase 2, Flights 1-5	Phase 2, Flights 14-18	All Phase 2 Flights	All Phase 3 Flights	All Phase 4 Flights
LRLB Birds	281.8 ± 141.7	189.1 ± 35.4	223.5 ± 86.7	218.4 ± 56.5	246.7 ± 90.1	227.6 ± 65.9	268.5 ± 159.7	185.9 ± 30.6
RLRB Birds	310.9 ± 216.1	202.0 ± 51.6	245.3 ± 130.8	253.9 ± 45.3	208.1 ± 50.52	223.9 ± 48.3	317.7 ± 463.6	210.0 ± 42.3
Controls	199.8 ± 71.1	195.6 ± 27.5	185.8 ± 43.4					

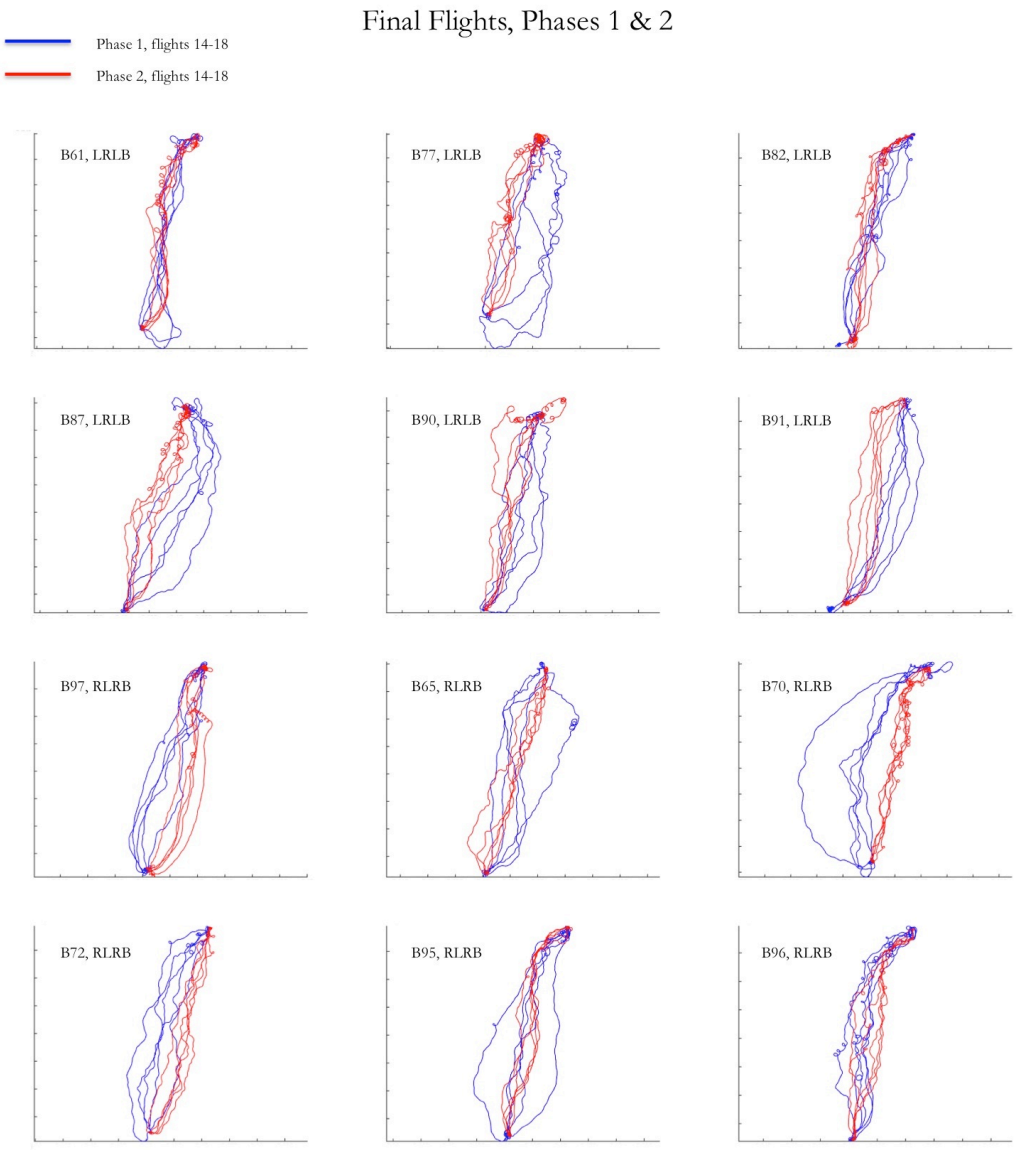


Figure S1. All birds' end-of-phase flights

This figure shows the traces of the last five flights in each of Phases 1 (blue) and 2 (red) for each of the 12 birds in the study. As can be seen, the flights of Phase 2 are generally flown over a different route from that flown during Phase 1. Neither group displayed a tendency to 'settle' back to their Phase 1 route in Phase 2.

References

1 Rogers, L. J., Vallortigara, G. & Andrew, R. J. *Divided brains: the biology and behaviour of brain asymmetries*. (Cambridge University Press, 2013).

2 Holland, R. A. The role of visual landmarks in the avian familiar area map. *Journal of Experimental Biology* **206**, 1773-1778 (2003).

3 Wallraff, H. G. Pigeon homing from unfamiliar areas: an alternative to olfactory navigation is not in sight. *Communicative & integrative biology* **7**, 6929-6943 (2014).

4 Wiltschko, R. & Wiltschko, W. Avian navigation: from historical to modern concepts. *Animal behaviour* **65**, 257-272 (2003).

5 Gagliardo, A. Forty years of olfactory navigation in birds. *The Journal of experimental biology* **216**, 2165-2171 (2013).

6 Schmidt-Koenig, K. The sun compass. *Experientia* **46**, 336-342 (1990).

7 Guilford, T. & Taylor, G. K. The sun compass revisited. *Animal behaviour* **97**, 135-143 (2014).

8 Wiltschko, W. & Wiltschko, R. Magnetic orientation and magnetoreception in birds and other animals. *Journal of Comparative Physiology A* **191**, 675-693 (2005).

9 Guilford, T. & Biro, D. Route following and the pigeon's familiar area map. *The Journal of experimental biology* **217**, 169-179 (2014).

10 Biro, D., Meade, J. & Guilford, T. Familiar route loyalty implies visual pilotage in the homing pigeon. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 17440-17443 (2004).

11 Biro, D., Meade, J. & Guilford, T. Route recapitulation and route loyalty in homing pigeons: pilotage from 25 km? *Journal of Navigation* **59**, 43-53 (2006).

12 Gagliardo, A., Ioalé, P. & Bingman, V. P. Homing in pigeons: the role of the hippocampal formation in the representation of landmarks used for navigation. *The Journal of neuroscience* **19**, 311-315 (1999).

13 Meade, J., Biro, D. & Guilford, T. Homing pigeons develop local route stereotypy. *Proceedings of the Royal Society B: Biological Sciences* **272**, 17 (2005).

14 Güntürkün, O., Stüttgen, M. C. & Manns, M. Pigeons as a model species for cognitive neuroscience. *e-Neuroforum*, 1-7 (2014).

15 Prior, H. Lateralization of spatial orientation in birds. *Behavioural and morphological asymmetries in vertebrates (eds Malashichev Y., Deckel AW)*, 75-85 (2006).

16 Pecchia, T., Gagliardo, A., Filannino, C., Ioalè, P. & Vallortigara, G. in *Behavioral Lateralization in Vertebrates* 107-124 (Springer, 2013).

17 Gagliardo, A. *et al.* Bilateral participation of the hippocampus in familiar landmark navigation by homing pigeons. *Behavioural brain research* **136**, 201-209 (2002).

18 Gagliardo, A. *et al.* Hippocampus and homing in pigeons: left and right hemispheric differences in navigational map learning. *European Journal of Neuroscience* **13**, 1617-1624 (2001).

19 Gagliardo, A., Vallortigara, G., Nardi, D. & Bingman, V. P. A lateralized avian hippocampus: preferential role of the left hippocampal formation in homing pigeon sun compass-based spatial learning. *European Journal of Neuroscience* **22**, 2549-2559 (2005).

20 Manns, M. & Römling, J. The impact of asymmetrical light input on cerebral hemispheric specialization and interhemispheric cooperation. *Nature communications* **3**, 696 (2012).

21 Karten, H. J. & Hodos, W. Telencephalic projections of the nucleus rotundus in the pigeon (Columba livia). *Journal of Comparative Neurology* **140**, 35-51 (1970).

22 Voneida, T. J. & Mello, N. K. Interhemispheric projections of the optic tectum in pigeon. *Brain, behavior and evolution* **11**, 91-108 (1975).

23 Manns, M. & Ströckens, F. Functional and structural comparison of visual lateralization in birds–similar but still different. *Frontiers in psychology* **5** (2014).
24 von Fersen, L. & Güntürkün, O. Visual memory lateralization in pigeons. *Neuropsychologia* **28**, 1-7 (1990).
25 Folta, K., Diekamp, B. & Güntürkün, O. Asymmetrical modes of visual bottom-up and top-down integration in the thalamic nucleus rotundus of pigeons. *The Journal of neuroscience* **24**, 9475-9485 (2004).
26 Andrew, R. J., Mench, J. & Rainey, C. Right-left asymmetry of response to visual stimuli in the domestic chick. *Analysis of visual behaviour*, 225-236 (1982).
27 Freeman, R., Mann, R., Guilford, T. & Biro, D. Group decisions and individual differences: route fidelity predicts flight leadership in homing pigeons (Columba livia). *Biology letters* **7**, 63-66 (2011).
28 Prior, H., Wiltschko, R., Stapput, K., Güntürkün, O. & Wiltschko, W. Visual lateralization and homing in pigeons. *Behavioural brain research* **154**, 301-310 (2004).
29 Gagliardo, A., Odetti, F. & Ioalè, P. Factors reducing the expected deflection in initial orientation in clock-shifted homing pigeons. *The Journal of experimental biology* **208**, 469-478 (2005).
30 Ulrich, C. *et al.* Left-hemispheric superiority for visuospatial orientation in homing pigeons. *Behavioural brain research* **104**, 169-178 (1999).
31 Prior, H., Lingenauber, F., Nitschke, J. & Güntürkün, O. Orientation and lateralized cue use in pigeons navigating a large indoor environment. *Journal of Experimental Biology* **205**, 1795-1805 (2002).
32 Güntürkün, O. Lateralization of visually controlled behavior in pigeons. *Physiology & behavior* **34**, 575-577 (1985).

Chapter 3

Swapping Mallards: Monocular Imprinting in Ducklings

Antone Martinho III & Alex Kacelnik

Under review for Animal Behaviour.

"Then lett us drink and dance a Galliard
in ye Remembrance of ye Mallard,
And as ye Mallard doth in Poole,
Lett's dabble, dive, & duck in Boule.
Hough the bloud of King Edward,
By ye bloud of King Edward,
It was a swapping, swapping mallard!"

The Mallard Song

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.

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,[1,2] 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.[2-4] 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 [5], 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 [6]. Incomplete contralateral visual input has been demonstrated in local area homing in pigeons (*Columba livia*),[7] and interhemispheric transfer of pattern discrimination fails in chickens (*Gallus gallus*),[8] though in the latter case, reinforcement with food enabled previously failed transfer.[9] 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.[3,10] 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.[1,11-13]

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.[14-19] In its most characteristic natural form, imprinting manifests as a strong attraction by which young nidifugous birds tightly follow their mother,[18] 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,[16] 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.[20] Imprints may be formed for multiple stimuli to which the chick is exposed during the sensitive period,[21] 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.[21] Imprints can be formed of both visual and auditory stimuli,[22] and maternal auditory calls can enhance the strength of imprinted response to a visual stimulus.[23]

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.[24] 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.[24] 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.

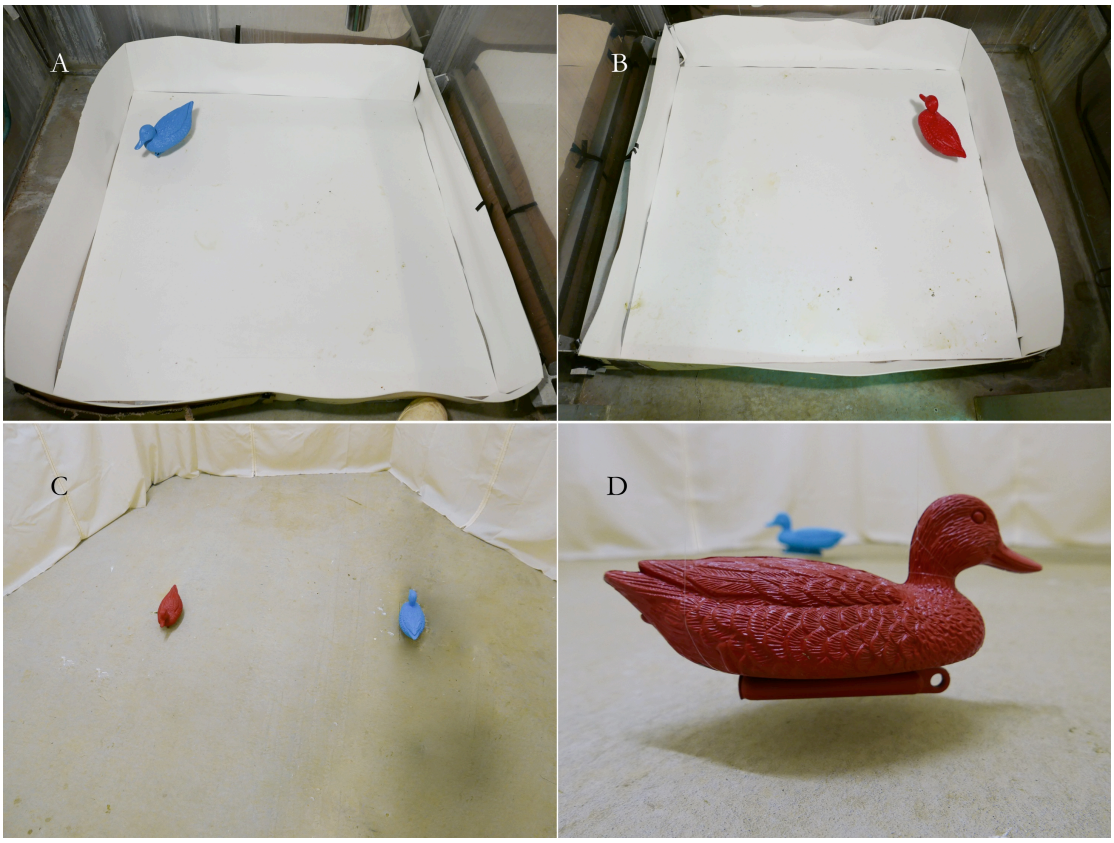


Figure 1. Training and Testing Chambers

(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 line to hang approximately five centimeters above the floor.

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,[21] as the highest sensitivity for imprinting has been shown to occur between thirteen and forty hours following hatching.[18] 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.[25] 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 [26] 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.[27] 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[18] 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.[28,29]

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.

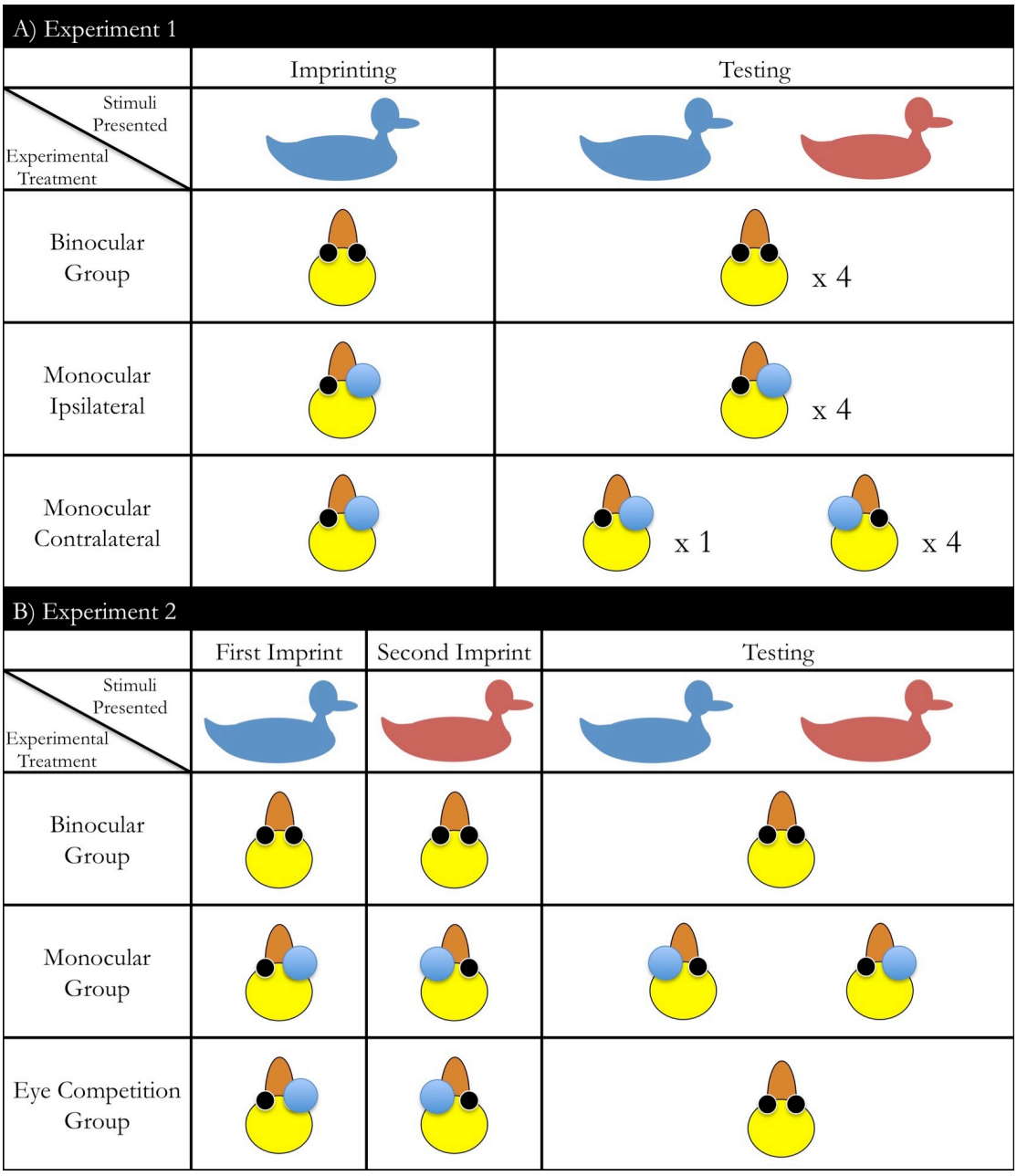


Figure 2. Schematic of Experimental Treatments

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.

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[30] in R.[31] 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.

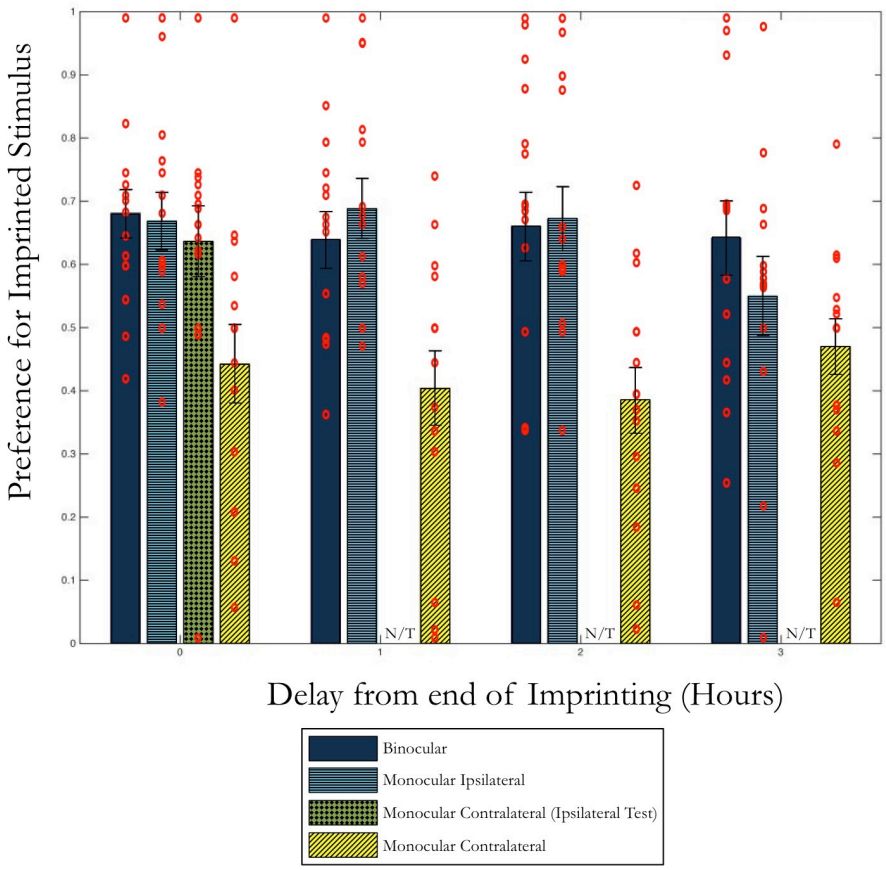


Figure 3. Stimulus preference (± standard error) during testing

Binocular (solid blue) and monocular ipsilateral (horizontally lined teal) groups showed a significant preference for their Imprinted Stimulus during testing, as did monocular contralateral birds during their initial, ipsilateral test (checkerboard green). The monocular contralateral group showed no significant preference during subsequent contralateral tests (diagonally lined yellow). 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.

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,[32] including (in chickens) in an imprinting context.[1] 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.

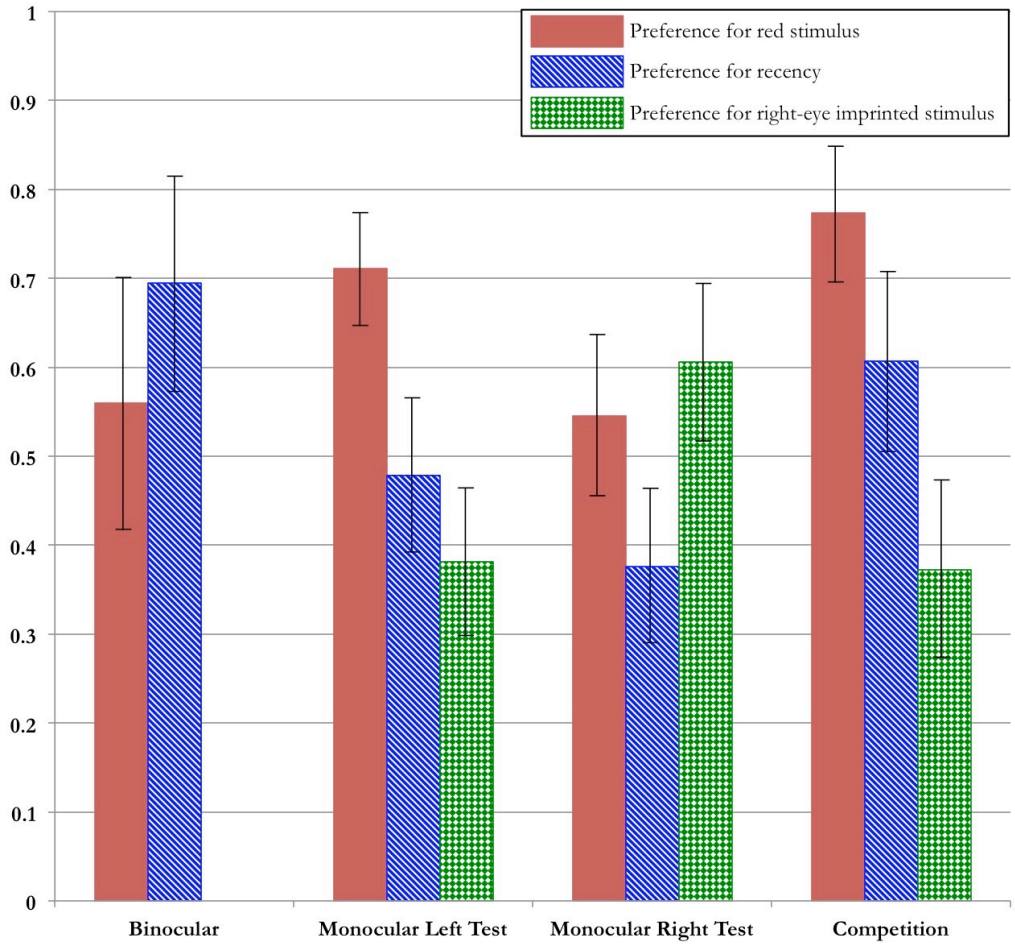


Figure 4. Stimulus preference (± standard error) during testing in Experiment 2

Mean preference is given for the red stimulus (solid red), the more recently imprinted stimulus (diagonally hashed blue) and the right-eye imprinted stimulus (checkerboard green), for each of the groups (twice for the Monocular Group, which was tested once with each eye). In contrast to Experiment 1, most birds preferred the red stimulus regardless of imprint or groups. The effect of recency preference was strong for the two groups (Binocular and Competition) that were tested binocularly, but not for the Monocular Group. The Monocular Group preferred the stimulus imprinted with the tested eye, while the Competition Group showed a slight left imprint preference. (The Binocular Group was not imprinted monocularly.)

The binocular group showed no significant reliable effects of recency, or preference for the more recently imprinted stimulus *(P* = 0.178, two-tailed, one sample t test), contrasting with the effect previously found in chickens when using multiple imprints in sequence.[21] The competition group showed no statistically significant preference for the stimuli imprinted with either eye *(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 ($P = 0.066$) 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,[3,7-10] and possibly marsh tits.[5,6,33]

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.[24] 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,[10,34] 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.[6,7,9] 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.[35,36] 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,[6] transitive inference,[10] and in homing route learning in pigeons.[7] 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.

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.

Acknowledgements

We thank the Oxford University Farm for allowing use of their hatchery facilities and ducklings, and offer special thanks to farm director Mike Gooding for approving the collaboration. We are extremely grateful to Mike Colley, poultry manager, for setting and managing the eggs, hatchery, and duckling care and husbandry, as well as for valuable input and advice on our methods. We thank Professor Pat Bateson for his comments and recommendations in developing our experimental procedures, and extend our gratitude to Isobel Watts and Lucy Taylor for assistance with our statistical analyses.

Supplementary Material

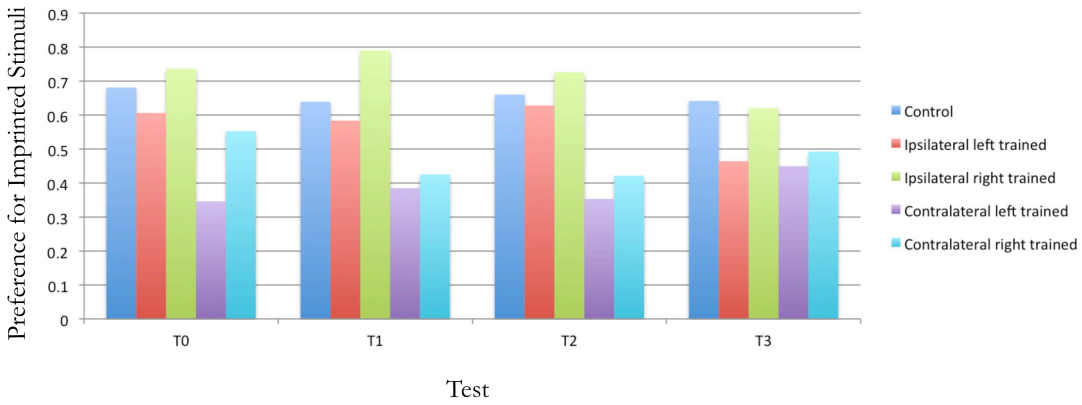


Figure S1. Stimulus Preference by eye in Experiment 1

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.

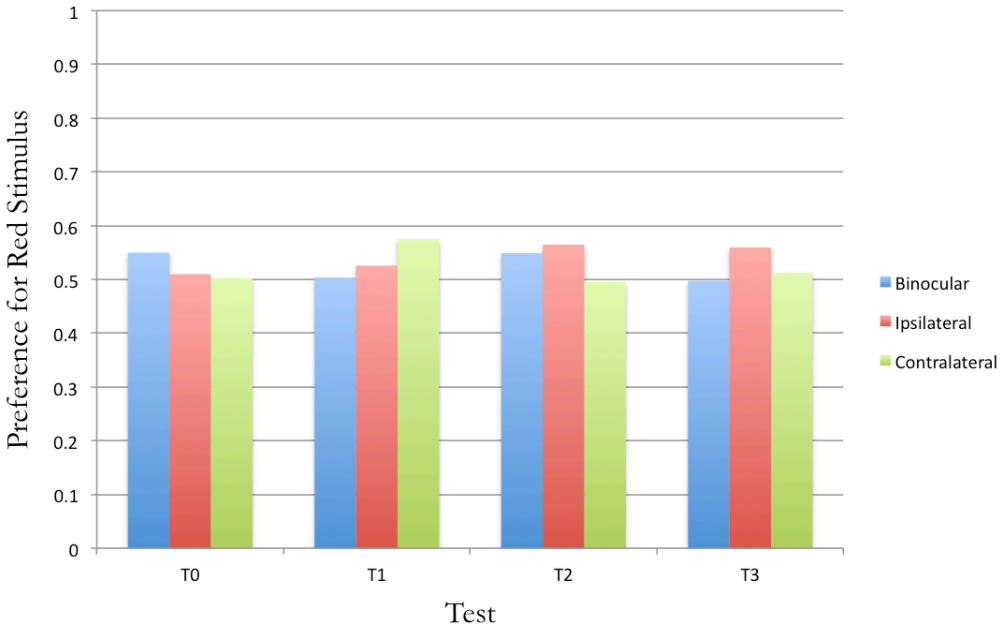


Figure S2. Stimulus Preference by colour in Experiment 1

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.

References

1 Rogers, L. J., Vallortigara, G. & Andrew, R. J. *Divided brains: the biology and behaviour of brain asymmetries*. (Cambridge University Press, 2013).

2 Parsons, C. H. & Rogers, L. J. Role of the tectal and posterior commissures in lateralization of the avian brain. *Behavioural brain research* **54**, 153-164 (1993).

3 Diekamp, B., Prior, H. & Güntürkün, O. Functional lateralization, interhemispheric transfer and position bias in serial reversal learning in pigeons (Columba livia). *Animal Cognition* **2**, 187-196 (1999).

4 Manns, M. & Ströckens, F. Functional and structural comparison of visual lateralization in birds–similar but still different. *Frontiers in psychology* **5** (2014).

5 Sherry, D. F., Krebs, J. R. & Cowie, R. J. Memory for the location of stored food in marsh tits. *Animal Behaviour* **29**, 1260-1266 (1981).

6 Clayton, N. Lateralization and unilateral transfer of spatial memory in marsh tits. *Journal of Comparative Physiology A* **171**, 799-806 (1993).

7 Martinho, A., Biro, D., Gagliardo, A., Guilford, T. & Kacelnik, A. Interhemispheric Transfer of Homing Routes in Pigeons. *Under Review for Proceedings of the Royal Society B* (2015).

8 Gaston, K. E. Lack of interocular transfer of pattern discrimination learning in chicks. *Brain research* **171**, 339-343 (1979).

9 Gaston, K. E. Interocular transfer of pattern discrimination learning in chicks. *Brain research* **310**, 213-221 (1984).

10 Manns, M. & Römling, J. The impact of asymmetrical light input on cerebral hemispheric specialization and interhemispheric cooperation. *Nature communications* **3**, 696 (2012).

11 Deng, C. & Rogers, L. J. Prehatching visual experience and lateralization in the visual Wulst of the chick. *Behavioural brain research* **134**, 375-385 (2002).

12 Horn, G. Imprinting—in search of neural mechanisms. *Trends Neurosci* **2**, 219-222 (1979).

13 Horn, G. & Johnson, M. H. Memory systems in the chick: dissociations and neuronal analysis. *Neuropsychologia* **27**, 1-22 (1989).

14 Lorenz, K. Imprinting. *Auk* **54**, 245-273 (1937).

15 Ramsay, A. O. & Hess, E. H. A laboratory approach to the study of imprinting. *The Wilson Bulletin*, 196-206 (1954).

16 Bateson, P. How do sensitive periods arise and what are they for? *Animal Behaviour* **27**, 470-486 (1979).

17 Ratner, A. M. & Hoffman, H. S. Evidence for a critical period for imprinting in Khaki Campbell ducklings (Anas platyrhynchos domesticus). *Animal Behaviour* **22**, 249-225 (1974).

18 Bateson, P. P. G. The characteristics and context of imprinting. *Biological Reviews* **41**, 177-217 (1966).

19 Bateson, P. P. G. Changes in chicks' responses to novel moving objects over the sensitive period for imprinting. *Animal Behaviour* **12**, 479-489 (1964).

20 Bateson, P. P. G. & Jaeckel, J. B. Chicks' preferences for familiar and novel conspicuous objects after different periods of exposure. *Animal Behaviour* **24**, 386-390 (1976).

21 Bolhuis, J. J. & Bateson, P. The importance of being first: a primacy effect in filial imprinting. *Animal Behaviour* **40**, 472-483 (1990).

22 Boyd, H. & Fabricius, E. Observations on the incidence of following of visual and auditory stimuli in naive mallard ducklings (Anas platyrhynchos). *Behaviour* **25**, 1-14 (1965).

23 Dyer, A. B. & Gottlieb, G. Auditory basis of maternal attachment in ducklings (Anas platyrhynchos) under simulated naturalistic imprinting conditions. *Journal of Comparative Psychology* **104**, 190 (1990).
24 Moltz, H. & Stettner, L. J. Interocular mediation of the following-response after patterned-light deprivation. *Journal of comparative and physiological psychology* **55**, 626 (1962).
25 Bateson, P. P. G. & Wainwright, A. A. P. The effects of prior exposure to light on the imprinting process in domestic chicks. *Behaviour*, 279-290 (1972).
26 Bateson, P. Brief exposure to a novel stimulus during imprinting in chicks and its influence on subsequent preferences. *Animal Learning & Behavior* **7**, 259-262 (1979).
27 Schaefer, H. H. & Hess, E. H. Color Preferences in Imprinting Objects1. *Zeitschrift für Tierpsychologie* **16**, 161-172 (1959).
28 Lickliter, R. & Gottlieb, G. Retroactive excitation: Posttraining social experience with siblings consolidates maternal imprinting in ducklings (Anas platyrhynchos). *Journal of Comparative Psychology* **101**, 40 (1987).
29 Lickliter, R. E. & Gottlieb, G. Social interaction with siblings is necessary for visual imprinting of species-specific maternal preferences in ducklings (Anas platyrhynchos). *Journal of Comparative Psychology* **99**, 371 (1985).
30 Bates, D., Maechler, M., Bolker, B. & S, W. lme4: Linear mixed-effects models using Eigen and S4. *Journal of Statistical Software* (2014).
31 R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria, 2013).
32 Skiba, M., Diekamp, B., Prior, H. & Güntürkün, O. Lateralized interhemispheric transfer of color cues: evidence for dynamic coding principles of visual lateralization in pigeons. *Brain and language* **73**, 254-273 (2000).
33 Clayton, N. S. & Krebs, J. R. Lateralization and unilateral transfer of spatial memory in marsh tits: are two eyes better than one? *Journal of Comparative Physiology A* **174**, 769-773 (1994).
34 Skiba, M., Diekamp, B. & Güntürkün, O. Embryonic light stimulation induces different asymmetries in visuoperceptual and visuomotor pathways of pigeons. *Behavioural brain research* **134**, 149-156 (2002).
35 Martinho, A., Burns, Z. T., von Bayern, A. M. P. & Kacelnik, A. Monocular Tool Control, Eye Dominance, and Laterality in New Caledonian Crows. *Current Biology* **24**, 2930-2934 (2014).
36 Troscianko, J., von Bayern, A. M. P., Chappell, J., Rutz, C. & Martin, G. R. Extreme binocular vision and a straight bill facilitate tool use in New Caledonian crows. *Nature communications* **3**, 1110 (2012).

Chapter 4

Monocular Tool Control, Eye Dominance, & Laterality in New Caledonian Crows

Antone Martinho III, Zackory T. Burns, Auguste M. P. von Bayern, & Alex Kacelnik

Originally published December 15, 2014, Volume 24.24, Current Biology. Published typesetting included in Appendix.

"You spend the first term at Oxford meeting interesting and exciting people and the rest of your time there avoiding them." – Cousin Jasper

Evelyn Waugh, *Brideshead Revisited*

Summary

Tool use, though rare, is taxonomically widespread, but morphological adaptations for tool use are virtually unknown.[1] We focus on the New Caledonian crow (NCC, *Corvus moneduloides*), which displays some of the most innovative tool related behavior among non-humans.[2-6] One of their major food sources is larvae extracted from burrows with sticks held diagonally[7] in the bill (Figure 1A-B), oriented with individual, but not species-wide, laterality.[8,9] Among possible behavioral[10] and anatomical adaptations for tool use,[5,11-15] NCCs possess unusually wide binocular visual fields (up to 60°), suggesting that extreme binocular vision may facilitate tool use.[5] Here we establish that during natural extractions, tool tips can only be viewed by the contralateral eye (Figure 2). Thus, maintaining binocular view of tool tips is unlikely to have selected for wide binocular fields; the selective factor is more likely to have been to allow each eye to see far enough across the mid-sagittal line to view the tool's tip monocularly.[5,16] Consequently, we tested the hypothesis that tool side preference follows eye preference, and found that eye dominance does predict tool laterality across individuals (Figure 3). This contrasts with humans' species-wide motor laterality and uncorrelated motor-visual laterality,[17] possibly because bill-held tools are viewed monocularly and move in concert with eyes, while hand-held tools are visible to both eyes and allow independent combinations of eye preference and handedness. This difference may affect other models of coordination between vision and mechanical control, not necessarily involving tools.

Results

View of the tool's tip

We modeled the geometry of natural food extractions combining morphological data,[15] and the structure of visual fields,[5] to compute the depths at which eyes see into foraging hollows as a function of hole diameter and probing distance (Figure 1, and Supplemental Experimental Procedures). According to this model (Figure 1C), the eye contralateral to the tool's tip sees the tip independently of a hollow's diameter, but the ipsilateral eye's maximum depth of view depends on hole diameter and distance between bill's tip and hole's opening. In the notation of Figure 1C, binocular viewing requires that $B \geq \lambda$.

We compared the set of parameters for which this inequality holds true in natural circumstances, using ecologically relevant hole dimensions from Bluff *et al.* (2010).[18] Sensitivity was examined by comparing three parameter ranges, one favoring binocularity, another monocularity, and a third interpolating between these (see Experimental Procedures).[19-21]

For each parameter set, we plotted two volumes in a space of tool's tip depth, hole diameter, and distance between bill and burrow's opening (Figure 2). One volume shows the region for which binocular vision of the tool's tip is possible, and the other the ecologically relevant space. The intersection between the two volumes shows the ecologically relevant area where binocular vision of tool tips is possible. Figure 2 shows this graphically and quantitatively in separate tables. Even assuming an extreme ecological range most favorable to binocularity, a majority of extractions occur in circumstances compelling monocularity. Given our conservative modeling, this intersection is likely to be much smaller, meaning that food extraction overwhelmingly involves monocular viewing of the tool's tip.

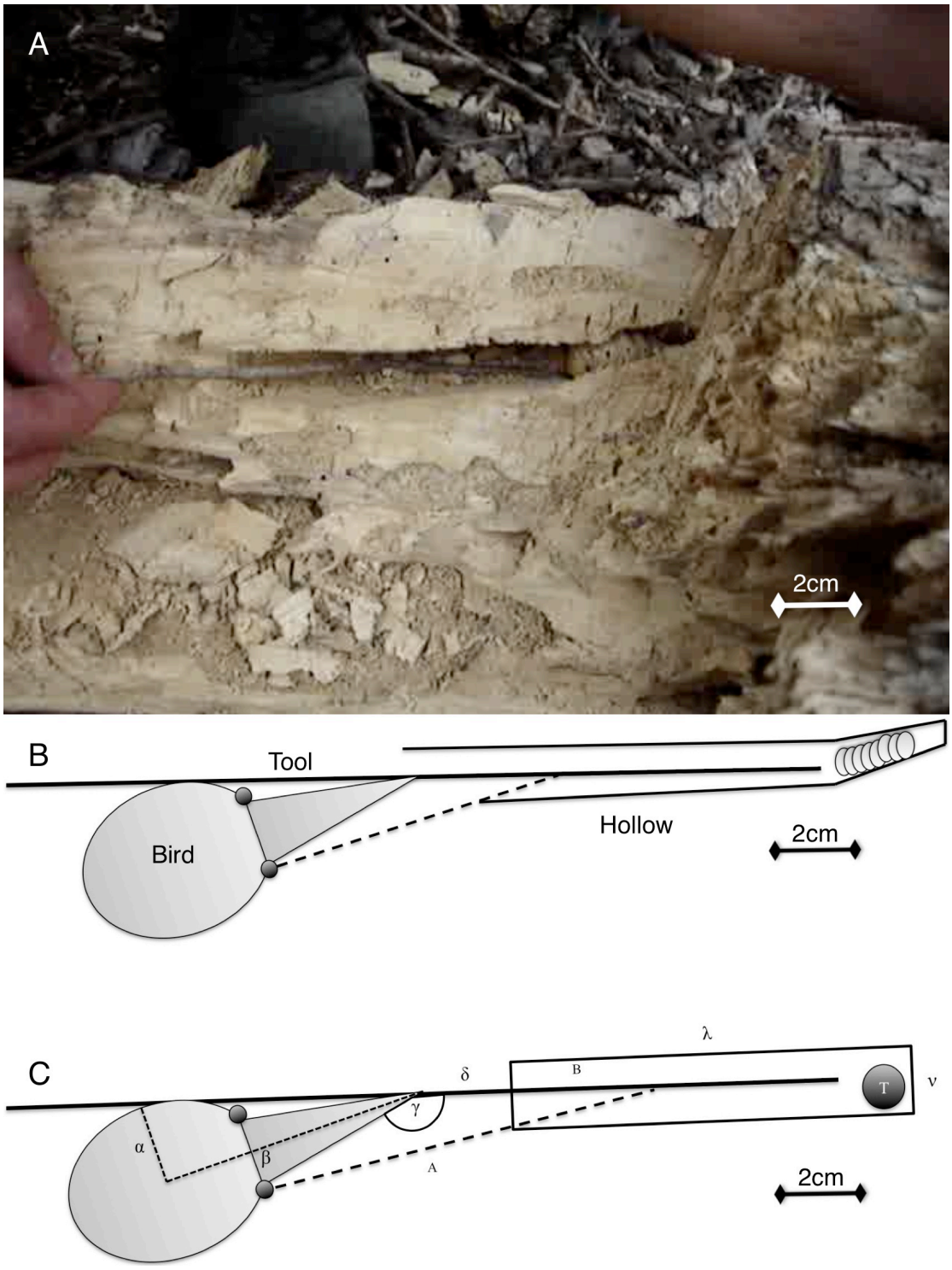


Figure 1. The geometry of vision and tool-use in New Caledonian crows.

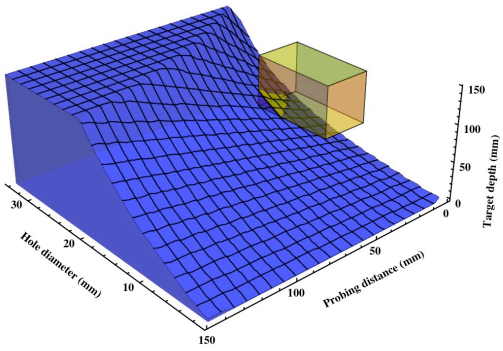
(A) Author AK probes a cross-sectioned larva burrow in a length of decaying candlenut wood. (B) A simplified rendering of the larval burrow shown in Figure 1A, showing from above how a crow probes for larvae using a lateralized tool grip. The angled opening of the burrow results in a varying target depth of 7-10 cm. (C) Top view of our model, generalized from Figure 1B with a

straight burrow and flat opening, showing a left-held tool being used to probe a cavity with a generalized target (T) at its bottom. The model identifies the obstructions of tool viewing by the eye on the side of the tool's tip. Using mean population morphology from Kenward *et al.* (2004) [15] for α and β, the angle of tool-use, γ, can be calculated and used to determine the angle and depth of the tool-tip's side eye's deepest line of sight into the hole, A. As 'δ' (distance between bill's tip and burrow opening) decreases, 'B,' (maximum depth of tool visibility) also decreases, non-linearly. Similarly, increases to the diameter of the hollow, ν, cause B to increase, while increases of target depth, λ, require a greater B for binocular viewing to be possible. As eye rotation and individual variation in the location of the eyes on the head cause varied inter-ocular distance, we placed the eyes at the widest point of the bill, thus modeling the inter-ocular distance as equivalent to bill width. This is the minimum possible inter-ocular distance and biases the model in favor of binocular vision by increasing the depth at which the eye on the side of the tool's tip can see into the burrow.

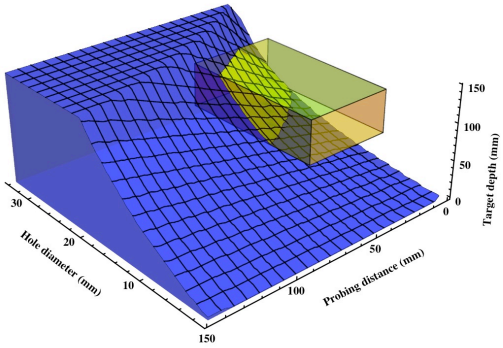
Figure 2. (Opposite) Ecological relevance of binocular and monocular vision.

Three sets of ecologically relevant ranges for hole diameter, target depth, and probing distance, one set of ranges favoring monocularity (A), one favoring binocularity (C), and an interpolation obtained by taking the midpoint of the range limits from each of the other two (B). For an explanation of how each of these ranges was derived, see Experimental Procedures. Depicted are the foraging burrow dimensions for which binocular viewing of target are possible (blue volume, which is the same across plots) and ecologically relevant burrow dimensions and probing distances (yellow prism, based on the relevant set of ranges from the table above each plot). The dark area of intersection shows the combinations of cavity dimensions and bill distances for which binocular viewing is possible. The size of this intersection is represented in the table as the percent of cases within the ecologically relevant yellow prism that are also within the binocular blue volume. As the plots reveal, this intersection is relatively small, amounting to less than half of the ecologically relevant conditions even under the parameters most favorable to binocularity. Thus, a majority portion of the crows' tool-use occurs under monocular conditions.

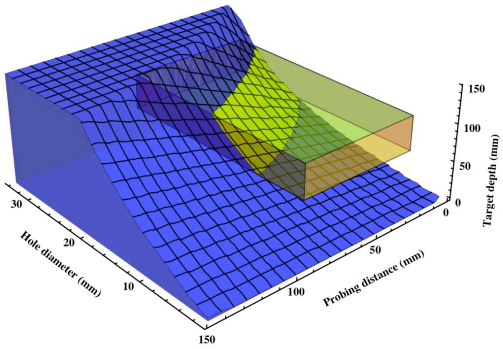
A	Monocular biased
Hole diameter	1.3 – 2.4 cm
Target depth	5.0 – 12.4 cm
Probing distance	0.0 – 3.0 cm
Percent binocular	2.5%



B	Interpolation
Hole diameter	0.95 – 2.9 cm
Target depth	4.65 – 10.9 cm
Probing distance	0.0 – 5.25 cm
Percent binocular	24.9%



C	Binocular biased
Hole diameter	0.6 – 3.4 cm
Target depth	4.3 – 9.3 cm
Probing distance	0.0 – 7.5 cm
Percent binocular	45.3%



Thus, the species' extremely wide visual fields may be an adaptation for tool use, but not through binocular control. Consistently with a general argument put forward by Martin (2010)[16] for bird visual fields in general, the wide overlap between left and right visual fields in NCCs may be a consequence of allowing each eye to see far enough across the mid-sagittal plane to include the tip of obliquely held tools. The NCCs' strong individual laterality of tool-use and lack of consistent species-wide bias may result from holding tools such that their tips fall in the field of the preferred eye. If this is true, eye preference should predict individual tool sidedness. We test this prediction by examining whether the eye preferred by individual NCCs to explore potential hollows in the absence of available tools predicts individual tool-holding sidedness.

Eye dominance and laterality

Nine out of 10 subjects completed an eye-dominance test, of which 4 were right- and 5 left-biased. All of them also completed a tool laterality test, of which 3 were right- and 6 left-biased. Eye preference reliably predicted tool sidedness (see Figure 3; $n = 9$, two-tailed binomial test, $p = .039$), with only one subject opposing the prediction (Figure 3 and Supplemental Data, Table S1).

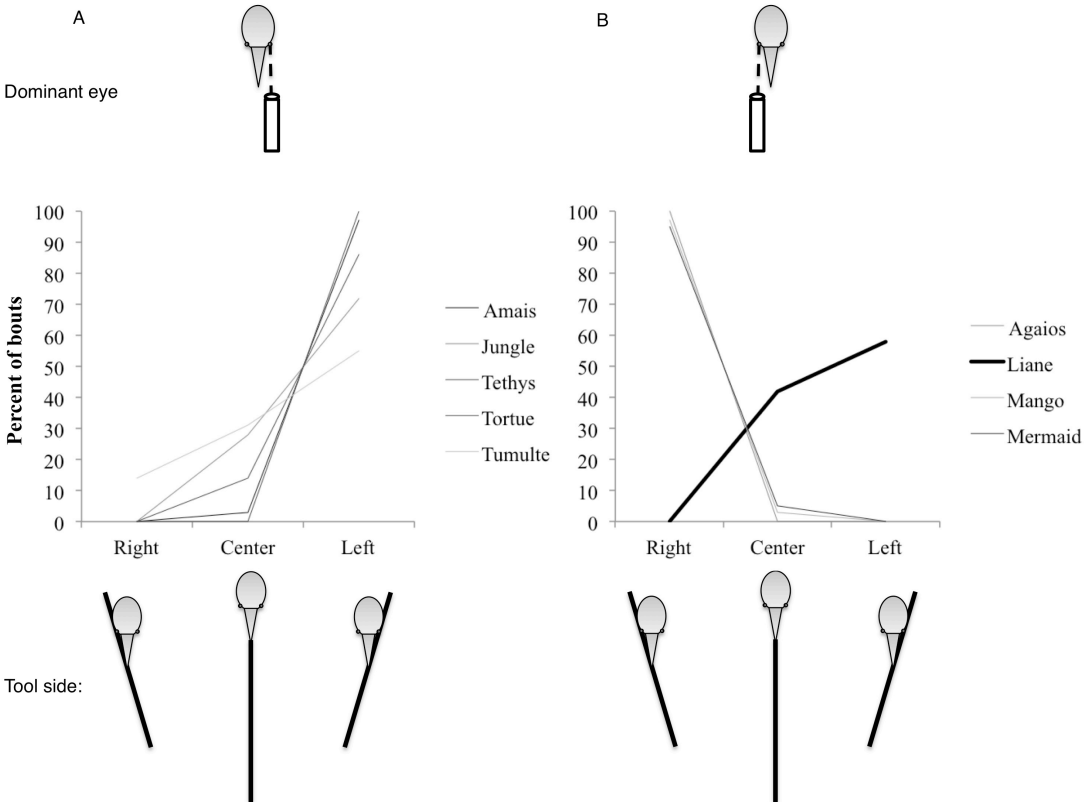


Figure 3. Percentages of lateralized tool bouts for (A) left-eye and (B) right-eye dominant individuals.

Each line represents an individual, showing the overall preference to hold the shaft of the tool on the same side as the dominant eye. 'Liane', the exception, is shown by the dark line in (B). Note her high percentage of center-held bouts.

Discussion

NCCs show strong individual lateralization of tool-use, display no detectable population-level bias ([8,9] and present data), have unusually wide overlap between the visual fields of both eyes, and prefer to hold their tools' working end on the side opposite to their preferred eye. By contrast, humans show species-level laterality of handedness and no correlation between the dominant eye and hand,[17,22] probably reflecting the difference between unity of movement between head and tools in crows, as compared to independence of movement between head and hands in humans.

Given that tool use is rarer than eye dominance –the latter affects multiple behavioral traits and taxa[23] – it is reasonable to infer that the causality goes from vision to tool sidedness rather than *vice versa*. The avian brain shows species-wide functional lateralization, and this may have been expected to cause consistent species-level laterality in eye dominance and tool use, but so far neither consistency has been found. Alternatively, eye dominance could be a consequence of individuals compensating for differences in quality of vision between their eyes. Such remedial dominance is unlikely to have species-wide consistency, but is consistent with eye dominance being causally related to tool use preference. Humans' eye dominance is about one third left and two thirds right, while handedness is roughly 90% right.[17] However, since humans and other primates manipulate tools with their hands, which can be moved independently of the head, differences between left and right eyesight do not need to constrain individuals' handedness. Crows instead are constrained to move tools and eyes in concert, and hence a random distribution of left-right eye preference probably leads to individual but not species-wide side bias. Further, eyesight-determined laterality in tool use may act against the evolution of species-wide hemispheric specialization for tool control.

This line of thinking may be relevant to other forms of laterality, constrained or not by joint movement of body parts. For instance, at least one species studied so far, the Japanese jungle crow, *Corvus macrorhynchos,* appears to have species-level laterality of footedness across tasks.[24] The contrast with bill-controlled tool use may reflect that footedness in birds, like handedness in humans, is unconstrained by side differences in eyesight.

Experimental and Modeling Procedures

Geometric model

Modeling the geometry of tool-assisted extractions in ecological circumstances required estimates of 6 parameters. Three describe anatomical traits (eye separation, bill and head length, and head width), and three are ecological variables that present greater uncertainty (dimensions of probing holes, depth at which the tool tip operates, and distance between the NCCs' bill tip and the hole's opening). Some potentially relevant correlations are poorly known, in particular the relation between prey and hole dimensions. For instance, Rutz *et al.* 2010[7] give the sizes of larva collected by humans in the NCCs habitat as 3.6 – 7.6 cm in length and 1.0 cm – 1.6 cm in girth, but it is not known how these sizes are represented in the crows' diet or the size of holes in which they reside. We used information in the supplementary materials of Bluff *et al.* (2010),[18] describing the dimensions of holes in which tools were left behind by NCCs. With this information we generated three sets of parameters, as indicated in Figure 2.

Operational tool tip depth (Target depth)

NC crows extract beetle larva predominantly by provoking them to bite the tool tip, so that both hole depth and position of the larva's head influence the operational location of the tool's tip. Bigger larvae reside in deeper burrows, but their greater length means that their heads are farther from the hole's bottom. As this correlation is unmapped, we used the hole depth quartiles provided by Bluff *et al.* (2010).[18] They provide two sets of quartiles, one for twig tools and one for leaf stem tools. The lower pair of middle quartiles (4.3 – 9.3 cm) comes from twig tools and favors binocularity, and the higher (5.0 – 12.4 cm), from leaf-stem tools, favors monocularity. Averaging the extremes of both ranges gave us our interpolation range, 4.65 – 10.9 cm.

Hole diameter

The hole openings described by Bluff *et al.* (2010)[18] had mean (±SD) minimum and maximum diameters of 1.392 ± 0.65 cm and 2.554 ± 1.25 cm respectively. For the sensitivity analysis favoring binocularity, we used a range of one standard deviation on either side of the mean of these diameters, estimating standard deviation as the square root of the sum of the two variances, giving a range of 1.97 ± 1.40 cm. This range includes both holes too narrow to contain a larva, and holes of greater diameter than the average NCC's head[15] which would decrease the use of tools, as the bird could easily access the prey with its bill.[25] We thus tightened our monocularity favoring range to span from the median minimum diameter, 1.3 cm, to the median maximum diameter, 2.4 cm, and held the median at 1.97 cm. The interpolation range was the midpoint between these two ranges.

Probing distance

As bill to hole opening distance increases, the possibility of binocular view of tool tips increases. Tool length is not a suitable guide for this parameter because tools are most frequently held at intermediate points and inserted at various depths, from zero at insertion time upwards, resulting in bill tip to hole opening distances often shorter than 2 cm. We used the 0 – 3 cm as the range favoring monocular viewing, and 0 – 7.5 cm as the range favoring binocular viewing, including the insertion distance of above average length tools. The interpolation range, 0 – 5.25 cm, selected was the midpoint of each value for these two ranges.

Subjects and housing

Subjects were 13 wild caught NCCs (7 females and 6 males), with experimental histories. They were housed in groups of 2 or 3 in outdoor aviaries (60 m^2) with indoor

enclosures (8m^2), with *ad libitum* food for 12 hours per day, and water at all times. Details of housing conditions can be found in von Bayern *et al.* (2009).[26] All experiments complied with German animal experimentation legislation (§7 Bundestierschutzgesetz).

Tool laterality test

Tool laterality was determined following Weir *et al.* (2004) [8], except where otherwise noted. We used a semi-circular tree stump section with two holes on its side, each 1.6 cm in diameter and 10 cm deep. A length of dowelling 0.3 cm in diameter and 20 cm long was furnished for use as a tool (Figure 4A). This doweling was longer than tools left behind in nature,[18] as longer tools result in a higher incidence of lateralized tool use.

Testing occurred before the usual food supply was placed in the indoor aviary each morning, *ca.* 11 hours after *ad libitum* food had been removed the previous night. The probing holes were baited with chilled mealworms, with an additional mealworm near the openings to attract the birds' attention. The surface of the apparatus was sprinkled with sawdust to approximate the cues left by NCCs' natural prey.[27] The log was placed in the enclosure approximately 10 cm from the center of a wall with the dowelling placed parallel to the wall, 10 cm in front of the apparatus. The test bird was then confined to the indoor enclosure and visually isolated from the outside aviary. Sessions were video-recorded from above the apparatus and lasted 30 minutes from the time of presentation.

Laterality of tool-use was scored in 'bouts' of tool use.[8] A new bout started if the crow changed tool grip or released the tool and moved its head before re-grasping it. Bouts were scored as 'right' or 'left' according to the cheek against which the non-functional end of the tool rested (Figure 4C) and 'centered' if the tool was held

straight. Tests were completed once the bird engaged in a minimum of 10 lateralized bouts. If the subject did not achieve this in one half hour, it was retested once and the bouts from both trials were pooled. To compute individual laterality we excluded center-held bouts, as did Weir *et al.* (2004).[8] The statistical significance of individual lateral bias was determined by a two-tailed binomial test against a random expectation of 50%.

Eye dominance test

Subjects that completed the tool laterality test were tested for eye dominance in the same enclosure. The apparatus was a rectangular wooden board (15.5 x 21 cm) with an opaque plastic tube 8.5 cm tall and 1.6 cm internal diameter protruding from its center (Figure 4B). The tube contained a darkling beetle larva (*Zophobas morio*), and the board was sprinkled with sawdust and mealworms to attract the crow's attention.[27] The apparatus was placed 10 cm from a wall's midpoint. The subject was confined to the indoor enclosure after placement of the apparatus. Tests were video recorded from above.

In the absence of potential tools, the bait was out of the bird's reach. Seven subjects made one look down the tube, of which six then abandoned the apparatus and one attempted to upturn it. The remaining two looked more than once and attempted to retrieve the food. We operationally defined eye dominance by the first 'look'. This normalized analysis across subjects, and controlled for the possibility that eye preference is expressed both in absolute use and order of eye use. A look was defined by one eye being placed directly above and within a bill's length of the tube's opening (Figure 4D), ensuring a direct and uninterrupted line of sight to the tube's bottom.

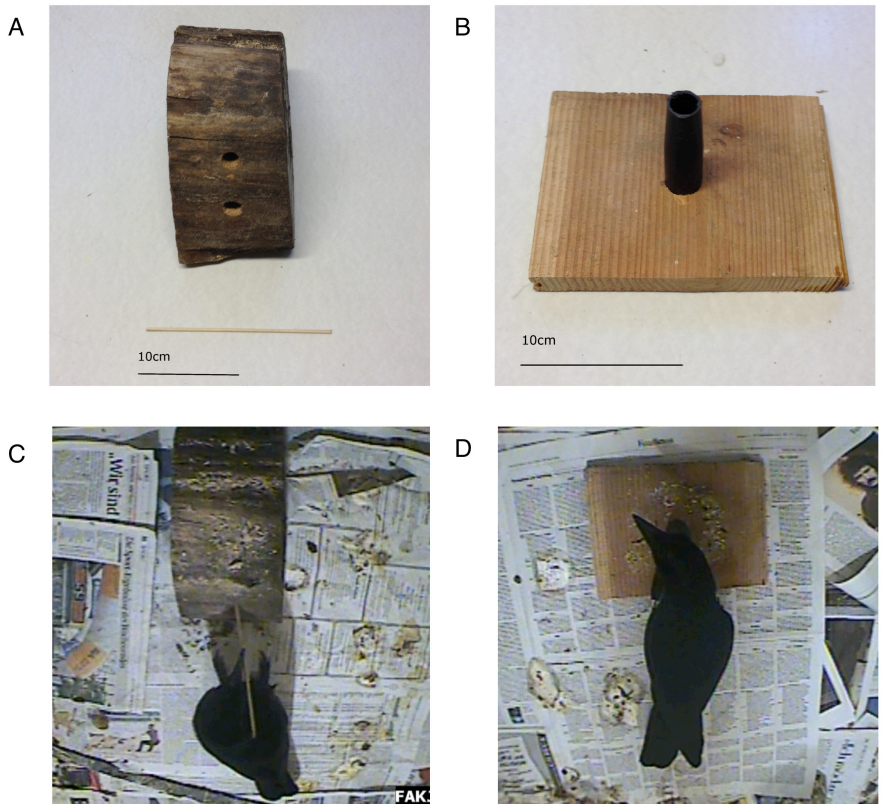


Figure 4. Experimental apparatus.

(A) Tool laterality probing apparatus and tool. Note the perpendicular placement of the tool to prevent approach bias. (B) Eye dominance apparatus. (C) Right-sided tool-use by 'Mango', viewed from above. (D) A right-eye 'look', with eye placed directly to the hole, by 'Liane', viewed from above.

Sessions lasted between 5 and 30 minutes, ending 5 minutes after the first look. Although the testing room was searched prior to tests to eliminate potential tools, one crow (*Agaios*) retrieved a previously cached tool and approached the apparatus with it before having looked. We removed the tool once the bird dropped it, and resumed testing. Each crow was tested until it looked once or failed to do so in 4 sessions. Four subjects were excluded from analysis because they didn't look at the apparatus.

Author Contributions

AM and ZB conceived and designed the study in collaboration with AvB and AK. Data were collected by AM and ZB in collaboration with AvB, who was also responsible for the NCC colony. AM performed analyses with support from ZB and AK. AM, ZB, and AK wrote and edited the manuscript, with input from AvB.

Acknowledgements

We thank the authorities of Province du Sud, Nouvelle Calédonie and Christian Rutz for help in sourcing our subjects. AM and ZB were supported by the Oxford Department of Zoology Graduate Research Fund. We are grateful to Berenika Moduszewska, Lauriane Rat-Fischer, Jasmin Werner, and Irmgard Neumair for their expertise in the care and handling of the crows and laboratory assistance. Kate Reynolds assisted with preparation of Figure 2. Our colleagues John Endler, Brian Rogers, Michael Haslam, Victoria Tobolsky, Jolyon Troscianko and Dora Biro made constructive comments on previous versions.

Supplemental Data

Table S1. Data record for all trials**.**

Tool laterality was assessed via two-tailed binomial test against an even chance, with all NC crows lateralized with $p < .001$. Of 9 birds, 8 consistently held a tool with the tip on the opposite side as their dominant eye. Individual notes: * completed tool laterality test in 2 sessions; ** completed eye dominance test in second session; *** failed to provide tool-laterality data; **** provided data on tool laterality but not eye dominance.

	Tool Laterality					**Eye Laterality**	
Bird	Sex	Left Bouts	Center Bouts	Right Bouts	Tool Laterality	First Look	Correlated?
Jungle	M	18	7	0	Left	Left	Yes
Liane	F	19	14	0	Left	Right	No
Amais**	F	72	2	0	Left	Left	Yes
Agaios	M	0	0	42	Right	Right	Yes
Mermaid**	F	0	2	40	Right	Right	Yes
Tortue	F	12	2	0	Left	Left	Yes
Mango	M	0	0	47	Right	Right	Yes
Tumulte*	F	41	23	11	Left	Left	Yes
Tethys*	F	18	0	0	Left	Left	Yes
Birds Excluded for Unsuccessful Trials							
Coralie***	F	9	0	0	Fail	NA	NA
Cpt. Hook****	M	18	9	0	Left	Fail	NA
Titan***	M	1	0	6	Fail	NA	NA
Mangroove***	M	0	0	0	Fail	NA	NA

Supplemental Experimental Procedures

R code for geometric analysis.

Script outputs a comma separated values (CSV) file giving the maximum depth of vision by the tip-ipsilateral eye for each pair of hole diameter and probing distance values within specified ranges. Furthermore, the spreadsheet contains a binary value signifying whether the maximum depth in which a crow can see is greater than each hole depth in the given range, and a binary value determining if that set of hole parameters is ecologically relevant.

```
# NC Crow Binocular Probing Model

rm(list = ls())

#bird parameters, from Kenward et al. (2004) all entered in mm.
head.width<-33.7 #widest point of head
head.length<-38.3 #length of head without bill
bill.length<-45.1 #length of bill
bill.width<-20.9 #width of bill at base
#diameter of probing hole = i
#distance from bill tip to hole opening = j
#depth of probing hole = k

# Function 'get.point' uses bird parameters inputted above to calculate
# depth of vision for ranges of variables i, j, k
get.point <- function(x) {
  i <- x[1]
  j <- x[2]
  k <- x[3]
  bill.angle<-(2*atan((head.width/2)/((head.length/2)+bill.length)))
  tool.angle<-(pi-bill.angle)
  bill.side<-(sqrt(((bill.width/2)^2)+(bill.length^2)))
  imag.tri.side<-(sqrt((j^2)+((i/2)^2)))
  imag.tri.ang<-atan((i/2)/(j))
  sca.tri.a<-tool.angle-imag.tri.ang
  sca.tri.A<-sqrt((bill.side^2)+(imag.tri.side^2)-(2*bill.side*imag.tri.side*cos(sca.tri.a)))
  sca.tri.b<-acos(((imag.tri.side^2)-(sca.tri.A^2)-(bill.side^2))/(-2*sca.tri.A*bill.side))
  viewing.angle<-(pi-((2*pi)-(.5*pi)-(tool.angle)-(sca.tri.b)))
  max.depth<-(.5*i*tan(viewing.angle))
  max.depth
  sub.df<-data.frame(max.depth = max.depth, diameter = i, distance = j, depth = k)
  sub.df
}
```

```
# Install library plyr to allow construction of dataframe and set library
install.packages("plyr")
library(plyr)

# Set ranges for i, j, and k in millimeters
grid<-expand.grid(i=seq(1,30,.5),j = seq(1,150,3),k=seq(1,150,3))

# Construct dataframe of parameters i, j, k, and max depth vision using get.point()
final.df <- ldply(1:length(grid[,1]), function(x) get.point(grid[x,]))

# Assign dataframe column names
# max.depth gives maximum depth of vision for shaft-contralateral eye
# for given diameter and distance
names(final.df)<-c("max.depth","diameter","distance","depth")

# Adds column, 0 if binocular viewing is possible, 1 if not possible
# by comparing depth and max.depth
final.df$bm<-ifelse(final.df$max.depth >= final.df$depth, 0, 1)

# Adds column that determines if point is in ecologically relevant ranges,
# ranges can be inputted bellow in millimeters
# 1 it they are, 0 if not
final.df$norm<-ifelse(final.df$diameter>=13 & final.df$diameter<=26,
                ifelse(final.df$depth>=60 & final.df$depth<=140,
                     ifelse(final.df$distance>=0 & final.df$distance<=100,1,0),0),0)

# Counts cases of monocular and binocular viewing within natural range
# Number monocular
mono<-sum(final.df$bm==1 & final.df$norm==1)
# Number Binocular
bino<-sum(final.df$bm==0 & final.df$norm==1)

# Percentage of natural cases in which binocular viewing is possible
(bino/(mono+bino))*100

# Writes CSV of data set
write.csv(final.df,"data.csv")
```

References

1 Shumaker, R. W., Walkup, K. R. & Beck, B. B. *Animal tool behavior: the use and manufacture of tools by animals*. (JHU Press, 2011).

2 Hunt, G. R. Manufacture and use of hook-tools by New Caledonian crows. *Nature* **379**, 249-251 (1996).

3 Hunt, G. R. & Gray, R. D. Diversification and cumulative evolution in New Caledonian crow tool manufacture. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**, 867-874 (2003).

4 McGrew, W. C. Is primate tool use special? Chimpanzee and New Caledonian crow compared. *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**, 20120422 (2013).

5 Troscianko, J., von Bayern, A. M. P., Chappell, J., Rutz, C. & Martin, G. R. Extreme binocular vision and a straight bill facilitate tool use in New Caledonian crows. *Nature communications* **3**, 1110 (2012).

6 Rutz, C. & St Clair, J. J. H. The evolutionary origins and ecological context of tool use in New Caledonian crows. *Behavioural processes* **89**, 153-165 (2012).

7 Rutz, C. *et al.* The ecological significance of tool use in New Caledonian crows. *Science* **329**, 1523-1526 (2010).

8 Weir, A. A. S., Kenward, B., Chappell, J. & Kacelnik, A. Lateralization of tool use in New Caledonian crows (Corvus moneduloides). *Proceedings of the Royal Society of London. Series B: Biological Sciences* **271**, S344-S346 (2004).

9 Rutledge, R. & Hunt, G. R. Lateralized tool use in wild New Caledonian crows. *Animal Behaviour* **67**, 327-332 (2004).

10 Kenward, B., Weir, A. A. S., Rutz, C. & Kacelnik, A. Behavioural ecology: Tool manufacture by naive juvenile crows. *Nature* **433**, 121-121 (2005).

11 Delacour, J. *Guide des oiseaux de la Nouvelle-Calédonie et de ses dépendances*. (Delachaux et Niestlé SA, 1966).

12 Jollie, M. Phylogeny of the species of Corvus. *Biologist* **60**, 73-108 (1978).

13 Goodwin, D. Crows of the World., London. *British Museum* (1986).

14 Doughty, C., Day, N. & Plant, A. *Birds of the Solomons, Vanuatu & New Caledonia*. (Christopher Helm, 1999).

15 Kenward, B., Rutz, C., Weir, A. A. S., Chappell, J. & Kacelnik, A. Morphology and sexual dimorphism of the New Caledonian crow Corvus moneduloides, with notes on its behaviour and ecology. *Ibis* **146**, 652-660 (2004).

16 Martin, G. R. What is binocular vision for? A birds' eye view. *Journal of Vision* **9**, 14 (2009).

17 Bourassa, D. Handedness and eye-dominance: A meta-analysis of their relationship. *Laterality: Asymmetries of Body, Brain and Cognition* **1**, 5-34 (1996).

18 Bluff, L. A., Troscianko, J., Weir, A. A. S., Kacelnik, A. & Rutz, C. Tool use by wild New Caledonian crows Corvus moneduloides at natural foraging sites. *Proceedings of the Royal Society B: Biological Sciences* **277**, 1377-1385 (2010).

19 Wickham, H. The split-apply-combine strategy for data analysis. *Journal of Statistical Software* **40**, 1-29 (2011).

20 R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria, 2013).

21 Ligges, U., Maechler, M., Schnackenberg, S. & Ligges, M. U. Package 'scatterplot3d'. (2014).

22 Merrell, D. J. Dominance of eye and hand. *Human Biology* (1957).

23 Rogers, L. J. Development and function of lateralization in the avian brain. *Brain research bulletin* **76**, 235-244 (2008).

24 Izawa, E.-I., Kusayama, T. & Watanabe, S. Foot-use laterality in the Japanese jungle crow (Corvus macrorhynchos). *Behavioural processes* **69**, 357-362 (2005).
25 Taylor, A. H., Hunt, G. R. & Gray, R. D. Context-dependent tool use in New Caledonian crows. *Biology letters*, rsbl20110782 (2011).
26 von Bayern, A. M. P., Heathcote, R. J. P., Rutz, C. & Kacelnik, A. The role of experience in problem solving and innovative tool use in crows. *Current Biology* **19**, 1965-1968 (2009).
27 Troscianko, J. *Ecological, morphological, and behavioural aspects of tool-use in New Caledonian Crows* Ph.D. thesis, University of Birmingham, (2011).

Chapter 5

Same or different? Ducks Imprint on a Relational Concept

Antone Martinho III & Alex Kacelnik

Originally published July 15, 2016, Volume 353, Issue 6296, Science. Published typesetting included in Appendix.

"Though lofty aims
Catastrophe entail,
We'll gloriously succeed
Or nobly, fail!" – King Paramount

W. S. Gilbert, *Utopia Limited*

Abstract

The ability to identify and retain logical relations between stimuli and apply them to novel stimuli is known as relational concept learning. This has been demonstrated in a few animal species after extensive reinforcement training, and it reveals the brain's ability to deal with abstract properties. Here we show relational concept learning in newborn ducklings without reinforced training. Newly hatched domesticated mallards briefly exposed to a pair of objects that were either the same or different in shape or colour later preferred to follow pairs of new objects exhibiting the imprinted relation. Thus, even in a seemingly rigid and very rapid form of learning like filial imprinting, the brain operates with abstract, conceptual reasoning, a faculty often assumed to be reserved to highly intelligent organisms.

Introduction

Relational concepts, such as "same" and "different", have been demonstrated in a few animal species, typically following extensive training.[1,2] Relational concepts differ from other forms of categorical generalization. For instance, pigeons and bees can be trained to discriminate whether novel images contain humans or not,[3] or whether novel paintings are by Monet or Picasso[4] relying on the similarity between features of the training and novel stimuli. In relational concept learning, however, relative properties between training stimuli generate the relationship that has to be generalized to sets of novel stimuli.[5]

The relations of "same" and "different" have been used to study relational concept learning in a few primates and birds,[6] using a variety of protocols. For instance, in the Identity Matching to Sample (IMTS) protocol, an animal sees a sample stimulus and subsequently chooses between two test stimuli, one of which is identical to the sample. Reinforcement can be contingent on responding to the identical one ("same") or to the alternative ("different"). Honeybees can learn this discrimination and even transfer correct response to novel stimuli across sensory modalities (olfaction and visual texture).[7] The IMTS task requires learning the appropriate comparison between the working memory representation of the sample and the currently perceived test stimuli, but it does not require interpreting an abstract relationship between perceived items and then reapplying the same relation to discriminate between sets of novel objects.

A different procedure, which isolates relational learning, involves presenting more than one stimulus as a sample, and then selecting, from between various sets of stimuli, the set that has the same internal relation ("same" for sets of identical elements and "different" for sets of non-identical elements) as the sample set, a procedure called relational matching to sample (RMTS).[1,8] In RMTS what has to be retained is a relation

between stimuli, rather than a representation of a perceived stimulus. Primates,[9] pigeons [5], parrots,[10] and corvids,[1] have succeeded in such problems, and it has been cogently argued that this may indicate their possession of the ability to reason by analogy.[8]

Demonstrating capacity for both identity and relational matching to sample has so far used reinforcement, and often-extensive training, to allow subjects to infer the target relation. This contrasts with avian filial imprinting, a specialized form of unrewarded learning by which hatchlings acquire the ability to identify and then follow a parent or substitute parental object.[11-13] As could be expected from its biological adaptive significance, imprinting is one of the fastest and most reliable forms of learning.[14] In chickens and ducklings, high fidelity imprinting responses can be acquired in a few minutes of unrewarded exposure to a stimulus.[15]

Filial and sibling imprinting cannot be mediated just by snapshot representations of two-dimensional retinal images. Wood (2015) summarised the problem sharply: "Building an invariant object representation requires transforming patterns of retinal activity (view-specific information) into an abstract representation that is tolerant to retinal image changes and selective for a particular object (identity information)".[16] This is particularly relevant when considering that ducklings may benefit by recognizing not just their mother but also their group of siblings,[17] because broods may have different degrees of heterogeneity. Evidence showing that young birds are sensitive to abstract qualities of stimuli, both spontaneously and through imprinting, supports this view and shows that imprinting is far richer, as a learning phenomenon, than had originally been envisaged.[18-21] It is thus tempting to ask whether their abstraction abilities may extend to the more demanding phenomenon of relational concept representation, which has so far only been demonstrated through

extensive reinforcement in species with advanced intelligence. To this effect we modified the RMTS protocol to combine it with imprinting, as follows.

Following Bateson's[22] and Lickliter's[23] procedures for effective imprinting, we hatched domesticated mallard ducklings in the dark and kept them for 1 hour in a social group, with light, food, and water. They were then exposed for 25 minutes to a moving pair of sample objects, kept for a 30min retention interval in the dark, and presented for 10min with two novel pairs of moving objects (Figure 1). Sample stimuli within the pair shown in the imprinting phase were either equal to each other in both colour and shape, or different in one of these characteristics. Test stimuli in the preference test consisted of two stimuli pairs, composed of objects novel to the birds. In one test pair the objects were equal in colour and shape, and in the other they differed in either shape or colour (Figure 2). Detailed methods may be found in Supplementary Materials.

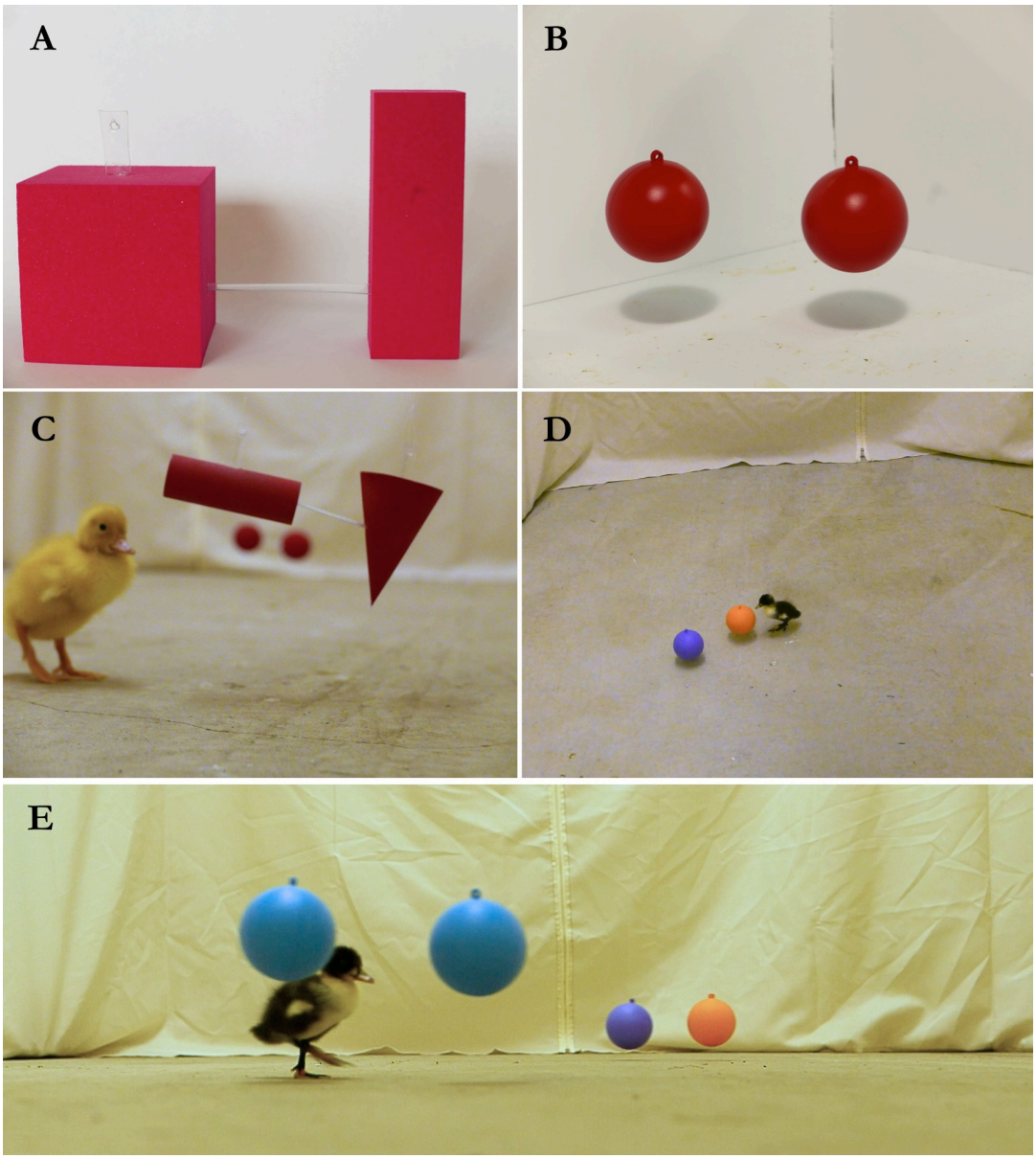


Figure 1. Imprinting and testing stimuli .

Newborn ducklings were first exposed to a pair of objects revolving about the centre of a training arena, then tested with two novel pairs of objects. (A) Example of a 'different shape' training stimulus pair. (B) Example of a 'same colour' training stimulus pair. Following this, the ducklings were tested for preference between two novel stimulus pairs revolving in apposition. (C) A duckling trained with the set shown in (A) demonstrates its preference for a novel 'different shape' stimulus over an also novel 'same shape' stimulus. (D) A duckling trained with the stimulus pair shown in (B) approaches a novel "different colour" stimulus pair – an "incorrect" response. (E) The same duckling later in the same trial correctly approaches and closely follows the novel 'same colour' stimulus.

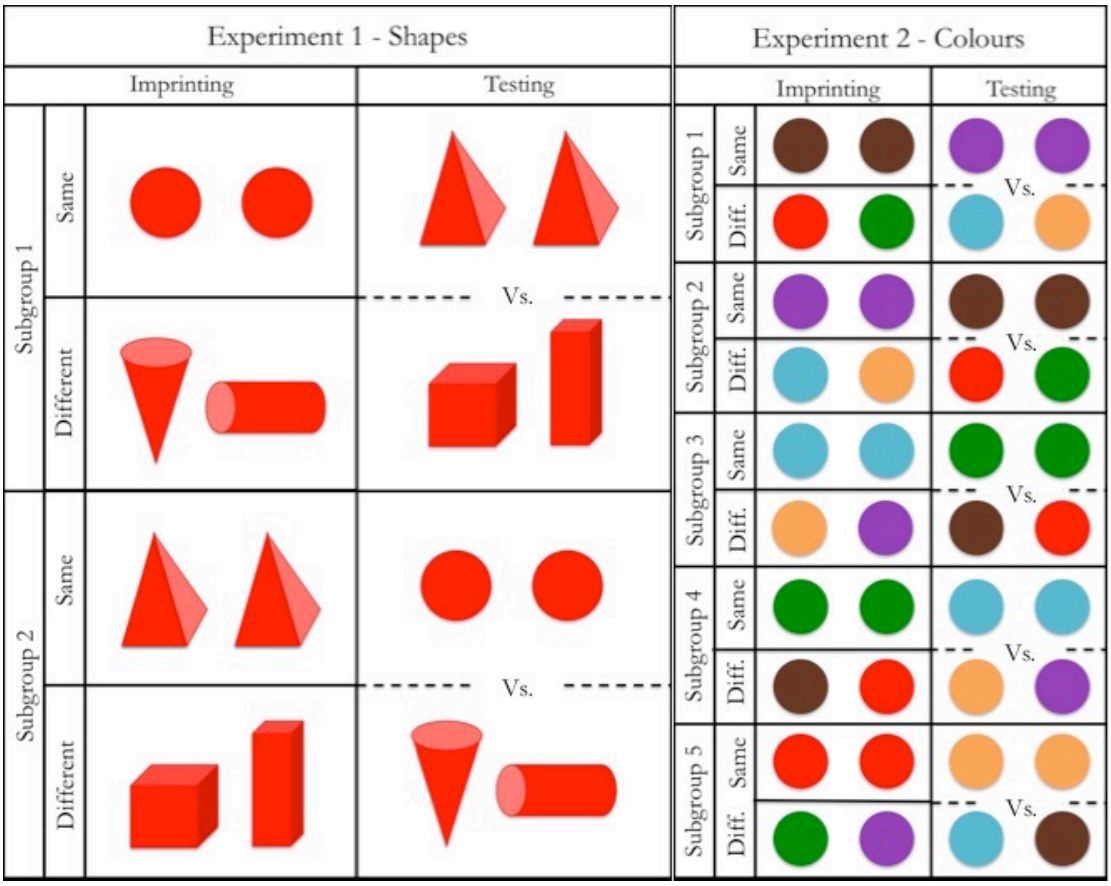


Figure 2. Experimental Stimulus Pairs

Experiment 1 tested for responses between red objects that could differ in shape and Experiment 2 for responses between spherical objects that could differ in colour, using the pairs illustrated here. In Experiment 1 members of Subgroup 1 were initially exposed to either two spheres or a pair of a cone and a cylinder, and then tested for preference between a pair of two pyramids and a pair formed by a prism and a cube, while members of Subgroup 2 were imprinted on pairs of either two pyramids or a prism and a cube and tested on preference between a pair of spheres and a pair formed by a cone and a cylinder. In Experiment 2 members of each subgroup were trained on either two identical spheres or a pair formed by two spheres of different colours, and all participants were tested for preference between two novel identical spheres and a pair formed by two novel, differing spheres.

Results

In Experiment 1, the imprinting phase stimulus pair consisted of two red solids, which were equal to each other in shape for group 'same' and different for group 'different' (n=36 for each group). The stimuli forming the two test pairs were also red, but one pair consisted of two novel, identical shapes and the other two novel, different shapes. In Experiment 2, the imprinting stimulus pair comprised two spheres, either of same or different colour (n=40 for each group) and test stimuli were also spherical but with novel colours, equal in one pair and different in the other.

To measure preference, the number of approaches undertaken by each duckling toward each test pair was scored twice, once by an independent scorer blind to each duckling's imprinting condition and to the study's hypothesis. Ducklings that were inactive during testing (fewer than five approaches) were excluded from analysis. Preferences were assessed via sign test, with sample size being the number of individual ducklings. Ducklings making more than half of their approaches toward a given stimulus were scored as having preferred it (See Methods in Supplementary Materials).

Figure 3 shows the preference results. In Experiment 1, 25 inactive ducklings were excluded; out of a total of 47 active ducklings, 32 preferred the pair bearing their imprinted shape relation (two-tailed binomial test, $p = 0.02$). In Experiment 2, 14 inactive ducklings were excluded; out of 66 active ducklings, 45 preferred the stimulus pairs bearing their imprinted colour relation (two-tailed binomial test, $p = 0.004$). Combining both results, out of 113 active ducklings, 77 preferred the relational concept, same or different, upon which they had imprinted (two-tailed binomial test, $p < 0.0001$).

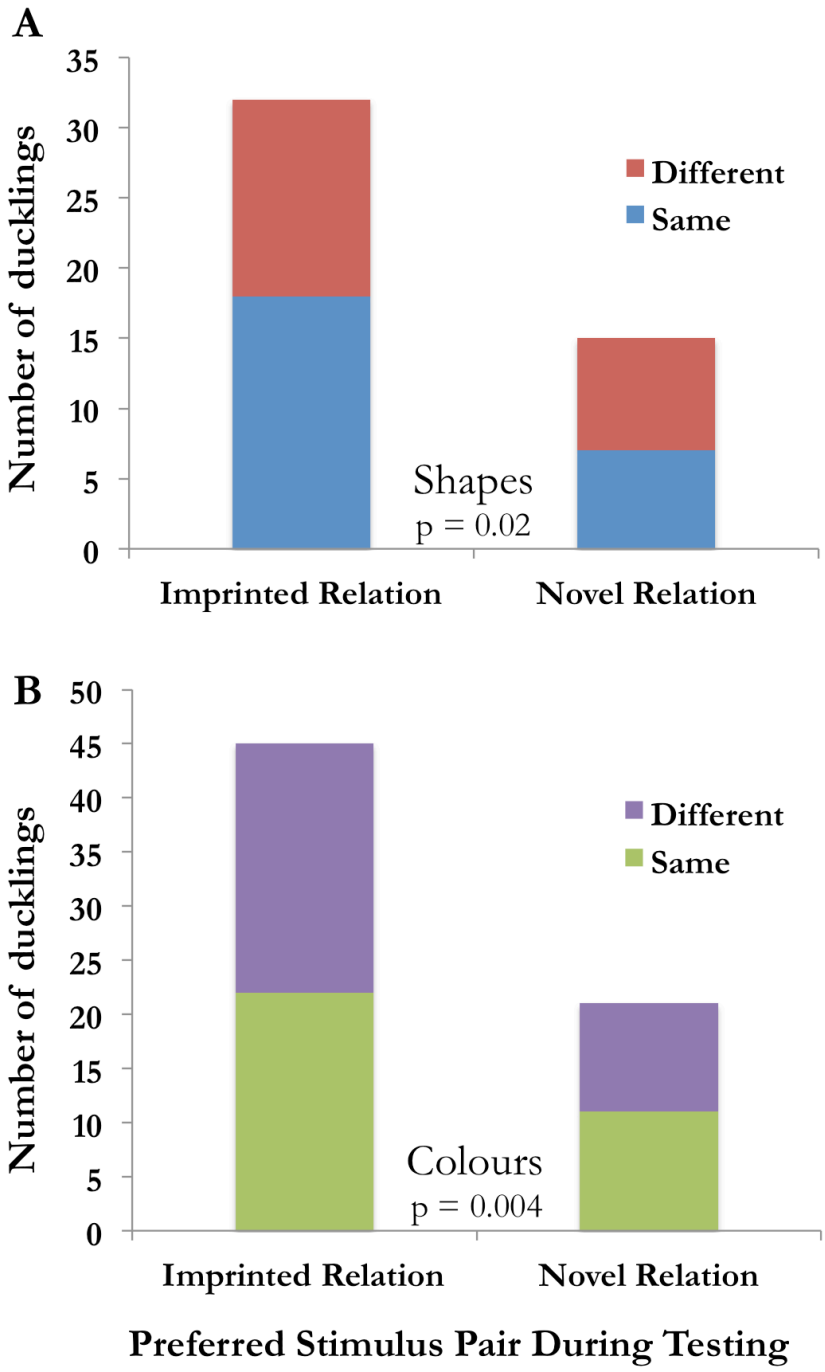


Figure 3. Ducklings' preferences for an imprinted relational concept

The number of subjects showing preference for the imprinted (left column) or alternative (right column) relation is shown for shape relations in Experiment 1 (A) and the same for colour relations in Experiment 2 (B). Ducklings preferred the imprinted relation in both shape and colour, regardless of whether the imprinted relation was 'same' or a 'different'.

Discussion

The accuracy of our ducklings was comparable to, or better than, reinforced relational concept discrimination in primates[24] and crows.[1] This finding supports a richer emerging view of the representation of information in the animal brain than is presently prevalent, in which even relatively simple learning systems do not process information just through the content of sensory signals but also by encoding higher-level, abstract aspects of stimulus analyses, already the target of neural network models designed to simulate such cognitive function.[25] The ducklings' performance indicates that their brains may be prepared, not just to respond differentially to certain visual inputs, such as scrambled objects containing species-specific elements like legs or heads or virtual points that move in a biologically plausible coordination,[20] but also to pick up abstract relational properties between elements of their sensory input and their characteristics.

For young precocious birds, having this competence makes biological sense. For a duckling critically dependent on proximity to its mother and siblings, defining the attachment stimulus configuration as a library of sensory inputs and logical rules increases the likelihood that the mother and sibling group will be identified with high fidelity in spite of considerable variations in how they are perceived. The rules that may define the imprinted attachment target are likely to extend beyond properties of a single object such as colour, shape, or symmetry, to include properties of object assemblies such as their informational entropy.[26]

Acknowledgments

We thank the Oxford University Farm for allowing us to use their ducklings for this work, and Poultry Manager Mike Colley for providing for duckling husbandry and technical help. Pat Bateson, Ed Wasserman and Hannah Smithson gave us valuable advice and recommendations regarding our methods and interpretation. We are also grateful to Emma Truswell, who assisted with some experimental trials, and Mateo Lewis, who acted as independent 'blind' scorer for data analysis. This research was funded with assistance from the University of Oxford Department of Zoology.

Materials and Methods may be found in Supplementary Materials. Video recordings of training and testing can be made available on request.

The experiment was devised jointly by AM and AK. Experimentation, data collection, and analysis were performed by AM with the advice and support of AK. The manuscript was written jointly by AM and AK and figures were prepared by AM and edited by AK.

Supplementary Materials (Materials and Methods)

Subjects

Subjects consisted of 154 newly hatched pekin (*Anas platyrhynchos domestica*) ducklings between 24 and 48 hours old. Seventy-two ducklings were used in Experiment 1 and eighty in Experiment 2. In each experiment, half of the ducklings were imprinted on a 'same' stimulus pair, and half on a 'different' stimulus pair (see Figure 2). Eggs were sourced from the University Farm in Oxford, UK, and incubated and hatched on-site. Ducklings were returned to this source upon completion of experiments. All experiments were approved by the University of Oxford Department of Zoology's Animal Welfare and Ethical Review Body.

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, ducklings remained in the dark chamber for approximately 24 hours to allow drying and to ensure imprinting could occur.[27] One hour before the commencement of experimentation, ducklings were moved to a brooding chamber with ad libitum food and water access illuminated from above with a 250W heat and light bulb to prime them for imprinting.[1,21] Ducklings were primed socially with up to three other ducklings to improve imprinting response.[22] Duckling selection we performed with brief lighting to ensure that only sufficiently dry and healthy ducklings were included; potential ducklings were placed on a brooder tray, and were included only if they performed several stable steps, with those not sufficiently dry or too young returned to the brooding chamber. All subsequent handling of ducklings during experimentation was performed in complete darkness, or illuminated by a faint green light[13] to prevent the formation of extraneous visual imprints.

Imprinting

Ducklings were imprinted on one visual stimulus pair composed of two three-dimensional solids approximately five to eight centimetres in their longest dimension. In Experiment 1, all stimuli were red, but could differ in shape, whilst in Experiment 2, all stimuli were spheres, but could differ in colour. In each experiment, half of the ducklings were imprinted on a stimulus pair composed of two stimuli of the same shape and colour, and the other half on two stimuli that differed in either shape (Experiment 1) or colour (Experiment 2). All stimuli used for imprinting and testing are shown in Figure 2. Stimulus objects were suspended 5cm from the imprinting chamber 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 two revolutions per minute, as mobile stimuli have been shown to elicit better responses.[14] Imprinting chambers were illuminated by a 100W incandescent light bulb, and consisted of a square with 1.1m sides, within which the stimulus rotated.

Imprinting was performed immediately following priming in the lighted chamber. Each chick was transferred to the imprinting chamber and exposed to its assigned imprinting stimulus pair (IS) for 25 minutes. The bird was then placed in a dark brooding chamber with *ad libitum* food and water access for 30 minutes. Ducklings were again brooded socially with up to three others to improve imprinting responses.[14]

Testing

The testing chamber consisted of an hexagonal enclosure (3.5m diagonal) surrounded by white curtains. Two novel stimulus pairs were suspended 5cm from the chamber floor from a boom, and rotated in apposition in a circle of diameter 1.75m, at the same speed as during imprinting. Neither of the testing pairs contained a stimulus

that the duckling had seen before. In Experiment 1, one pair was same-shaped and one was differently shaped. In Experiment 2, one stimulus pair was same-coloured, and one differently-coloured. As in imprinting, all Experiment 1 stimuli were red and all Experiment 2 stimuli were spheres.

Ducklings were placed under an opaque dome in the centre of the enclosure, and testing commenced when this dome was lifted via a pole from outside the enclosure. Each test lasted 10 minutes. Testing began 30 minutes after completion of imprinting.

Scoring

All testing was video recorded from above. During testing, the total number of positive and negative responses to both object pairs was recorded. A negative response was defined as either a movement directly away from a stimulus previously approached, or a movement evading the oncoming approach by a stimulus. Positive responses were counted so as to control for different patterns of movement between 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 an object pair were counted as positive responses, reflecting an imprinted duckling's tendency to approach imprinted, rather than novel stimuli.[13] When a duckling consistently followed a stimulus around its circular path, one positive response, and no more, was counted for each 90° of stimulus revolution, 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 per stimulus revolution. All tests were scored by AM and rescored by an independent observer

blind to the duckling's pre-exposure and the study's purpose. Correlation was assessed by Spearman's rank correlation coefficient, and the scores were highly correlated (Experiment 1: $R = 0.9367$, Experiment 2: $R = 0.9489$).

Ducklings were assigned a preference by sign test, with ducklings exhibiting more than 50% of approaches to one stimulus scored as having preferred that stimulus. We analysed the sensitivity of this method of assigning preference, finding that statistical significance was reduced when preference required one stimulus to command five or ten more approaches than the other, as opposed to just one. Our results remain significant for the shape modality ($p = 0.02$ for five, $p = 0.05$ for ten) and the combined shape and colour analysis ($p = 0.003$ for five, $p = 0.02$ for ten) regardless of which of these three criteria is used. For the colour modality alone, the results remain significant when preference required five more approaches to one stimulus than the other ($p = 0.05$), becoming non-significant for ten ($p = 0.2$).

References

1 A. Smirnova, Z. Zorina, T. Obozova, E. Wasserman, Crows spontaneously exhibit analogical reasoning. *Current Biology* **25**, 256-260 (2015).

2 E. A. Wasserman, Comparative cognition: Beginning the second century of the study of animal intelligence. *Psychological Bulletin* **113**, 211 (1993).

3 R. J. Herrnstein, D. H. Loveland, C. Cable, Natural concepts in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes* **2**, 285 (1976).

4 W. Wu, A. M. Moreno, J. M. Tangen, J. Reinhard, Honeybees can discriminate between Monet and Picasso paintings. *Journal of Comparative Physiology A* **199**, 45-55 (2013).

5 T. R. Zentall, M. Galizio, T. S. Critchfied, Categorization, concept learning, and behavior analysis: an introduction. *Journal of the experimental analysis of behavior* **78**, 237 (2002).

6 A. A. Wright, J. S. Katz, Mechanisms of same/different concept learning in primates and avians. *Behavioural Processes* **72**, 234-254 (2006).

7 M. Giurfa, S. Zhang, A. Jenett, R. Menzel, M. V. Srinivasan, The concepts of 'sameness' and 'difference'in an insect. *Nature* **410**, 930-933 (2001).

8 R. G. Cook, E. A. Wasserman, Learning and transfer of relational matching-to-sample by pigeons. *Psychonomic Bulletin & Review* **14**, 1107-1114 (2007).

9 E. A. Wasserman, J. Fagot, M. E. Young, Same–different conceptualization by baboons (Papio papio): The role of entropy. *Journal of Comparative Psychology* **115**, 42 (2001).

10 I. M. Pepperberg, Acquisition of the same/different concept by an African Grey parrot (Psittacus erithacus): Learning with respect to categories of color, shape, and material. *Animal Learning & Behavior* **15**, 423-432 (1987).

11 K. Lorenz, Der kumpan in der umwelt des vogels. *Journal of Ornithology* **83**, 289-413 (1935).

12 P. Bateson, How do sensitive periods arise and what are they for? *Animal Behaviour* **27**, 470-486 (1979).

13 P. Bateson, Brief exposure to a novel stimulus during imprinting in chicks and its influence on subsequent preferences. *Animal Learning & Behavior* **7**, 259-262 (1979).

14 P. P. G. Bateson, The characteristics and context of imprinting. *Biological Reviews* **41**, 177-217 (1966).

15 P. Bateson, J. B. Jaeckel, Chicks' preferences for familiar and novel conspicuous objects after different periods of exposure. *Animal Behaviour* **24**, 386-390 (1976).

16 J. N. Wood, Characterizing the information content of a newly hatched chick's first visual object representation. *Developmental science* **18**, 194-205 (2015).

17 E. H. Hess, D. B. Hess, Innate factors in imprinting. *Psychonomic Science* **14**, 129-130 (1969).

18 O. Rosa-Salva, L. Regolin, G. Vallortigara, Faces are special for newly hatched chicks: evidence for inborn domain-specific mechanisms underlying spontaneous preferences for face-like stimuli. Developmental Science 13, 565-577 (2010).

19 G. Vallortigara, L. Regolin, F. Marconato, Visually inexperienced chicks exhibit spontaneous preference for biological motion patterns. (2005).

20 L. Regolin, L. Tommasi, G. Vallortigara, Visual perception of biological motion in newly hatched chicks as revealed by an imprinting procedure. *Animal Cognition* **3**, 53-60 (2000).

21 R. Rugani, L. Regolin, G. Vallortigara, Imprinted numbers: newborn chicks' sensitivity to number vs. continuous extent of objects they have been reared with. *Developmental science* **13**, 790-797 (2010).

22 P. P. G. Bateson, A. A. P. Wainwright, The effects of prior exposure to light on the imprinting process in domestic chicks. *Behaviour*, 279-290 (1972).
23 R. Lickliter, G. Gottlieb, Retroactive excitation: Posttraining social experience with siblings consolidates maternal imprinting in ducklings (Anas platyrhynchos). *Journal of Comparative Psychology* **101**, 40 (1987).
24 J. S. Katz, A. A. Wright, J. Bachevalier, Mechanisms of same-different abstract-concept learning by rhesus monkeys (Macaca mulatta). *Journal of Experimental Psychology: Animal Behavior Processes* **28**, 358 (2002).
25 P. Bateson, G. Horn, Imprinting and recognition memory: a neural net model. *Animal Behaviour* **48**, 695-715 (1994).
26 T. R. Zentall, E. A. Wasserman, O. F. Lazareva, R. K. Thompson, M. J. Rattermann, Concept learning in animals. *Comp Cogn Behav Rev* **3**, 13-45 (2008).
27 J. J. Bolhuis, P. Bateson, The importance of being first: a primacy effect in filial imprinting. *Animal Behaviour* **40**, 472-483 (1990).

Chapter 6

Concluding Remarks: What is it Like to be a Bird?

Antone Martinho III

Unpublished Manuscript

"Never look for birds of this year in the nests of the last." – Don Quixote de la Mancha

Miguel De Cervantes, *Don Quixote*

Summary of Findings

With the only exception of eutherian mammals, vertebrate brains have the problem of integrating sensory input from two independent eyes so that the organism acts as a well-informed whole. This thesis set out to investigate how vertebrates' brains solve this problem. In eutherian mammals such as humans, this is solved by a fast flow of information between the higher centres of the brain, where information is analysed and decisions are made, via the *corpus callosum*, a thick commissure connecting the two hemispheres. Yet other vertebrates likely have a similar need for fast decision making processes, and have to do this without the main route for immediate information integration used by eutheria. This thesis began with the question of how a bird, living with brain hemispheres whose two visual systems are independent, manages information, and behaves as a coordinated whole. Beginning with Chapter 2 and 3, which investigated this question directly, the line of inquiry proceeded to Chapter 4, where some behavioural strategies of coping with a split-brain design were explored, and Chapter 5, attempting to determine what information actually is to an avian brain.

In Chapter 2, homing pigeons were tested for the effects of the neurological independence between the right and left avian visual pathways, taking advantage of their tendency to recapitulate homing routes consistently. Trained to return home from a given location with one eye occluded over the course of several days, they were then subsequently released a number of times from the same location with the other eye occluded, to determine if the route and performance established in the first phase was conserved. I found that whilst the first flights undertaken with the new eye were indeed generally more efficient and less divergent from the beeline than the first flights with the original eye, they did not constitute recapitulation, with pigeons both beginning and ending the second phase flying a route significantly different from that of the first phase. This may suggest a lack of complete access to the first phase route

information during the second phase, though the improved initial performance suggests that not all benefits of experience are lost upon switching eyes.

In Chapter 3, the same principle was investigated in a substantially different system. Ducklings were allowed to form a maternal imprint onto a blue or red duck decoy with one eye occluded, then tested for preference between the two decoys with the same or the opposite eye occluded. Through the use of imprinting, this protocol allowed the transfer of visual information to be tested within the first three hours following acquisition, a period after which some evidence has suggested that visual information transfer may occur.[1,2] Control ducklings imprinted and tested binocularly, or monocularly with the same eye during both training and testing showed a significant preference for the imprinted stimulus, whilst those tested with the eye 'naïve' to their imprinted stimulus performed no better than chance in terms of preference between the two stimuli, suggesting the absence of transfer during this three hour window. A second experiment directly followed, investigating whether one eye's visual system (the left or right) might dominate behaviour should the two systems conflict as to the identity of the imprinting substrate. Here, ducklings were allowed to imprint on both decoys, each with a different eye, then tested either once with each eye, or a single time binocularly. The ducklings that were tested once with each eye showed a weaker, but still noticeable preference for the stimulus imprinted upon with the tested eye, suggesting some cross-hemisphere interaction, but a general preservation of the autonomy of the two visual memories. The ducklings tested binocularly did not show a preference for one eye's stimulus over the other, indicating either a lack of dominance or sufficient mingling of the two visual systems' information for both stimuli to evoke the following response.

Chapter 4 turns, then, to investigate the behavioural strategies a bird might employ to ensure optimal behaviour, given this partial independence of each eye's

perspective and information. New Caledonian crows are known for their deft use of stick tools during foraging, often probing narrow, deep hollows for the larvae that comprise a substantial portion of their calorie supply.[3] In doing so, they show a within-individual, consistent preference for clasping the tool against either the right or left side of the bill and face,[4,5] a laterality often compared to handedness in primates. However, unlike some primates, there is no species-level preference for one limb over another, and furthermore, this preference of tool-holding side does not reflect a preference between two bilaterally symmetric appendages (the arms), but for a positional choice of the tool with respect to a single, medial structure (the neck and head). By assessing the crows' eye dominance in a non-tool-use peering task and comparing this with tool-holding laterality in a basic tool-use task, I determined that the crows hold the tool against the side of the head corresponding with their preferred eye for peering. Mathematical modelling of the geometry of a crow probing a narrow hollow with a stick-tool indicated that this preference is likely to reflect the crows' attempts to ensure that the dominant eye can maintain visual contact with the tool-tip even in deep hollows. In light of the results of Chapters 2 and 3, this would seem a highly adaptive behaviour. A crow holding a tool such that the non-dominant eye can see the tip would result, at least instantaneously, in the dominant eye's visual system remaining naïve with respect to the information gathered, likely a disadvantage for a dextrous task such a larva extraction.

Chapter 5 was initially conceived as a response to a problem raised by the results of Chapters 2 and 3 but it ventures towards areas I plan to investigate beyond this thesis. In both the ducklings and the pigeons, there is a clear independence of the two visual systems evidenced by the poor performance of subjects guided by their naïve eye in tasks for which training used the alternative eye; and in both cases, performance guided by a naïve eye is not entirely like that of a totally naïve animal –

not entirely surprising, as the animal can derive information from sources other than vision, which may inform both hemispheres. All of this raised the question of what exactly information is to an avian brain, and how information gathered in a visual protocol is represented in a bird's brain for subsequent reference. I thus explored whether imprinting may involve the acquisition of one or more abstract concepts defining the imprinting substrate, rather than the formation of a visual memory thereof. Ducklings were imprinted on a pair of stimuli that were the same or different in either colour or shape, and tested for whether they could apply these concepts – "sameness" and "difference" – to novel pairs of objects, following the pair that exemplified the imprinted concept, even if it visually did not resemble the training pair. I found that the ducklings could indeed imprint on a concept and identify it in a novel stimulus, with accuracy comparable to their more typical imprinting as in Chapter 3, and indeed higher than the accuracy of primates performing a similar conceptual task. This suggests that the ducklings can learn and deploy abstract concepts in a single training session and without reinforcement training, through imprinting.

Implications

Each of the threads within this thesis – laterality, hemispheric integration, and concept learning – brings with it its own implications, and these are discussed in detail in the individual chapters themselves. As a summed body, however, they may be able to inform our understanding, to paraphrase Thomas Nagel, of what it is like to be a bird. This construction is associated with controversial areas such a consciousness, experience, and animal ethics, but it need not only refer to such contested topics. Insight into the information processing necessary for survival, especially the realities affecting what information is available to contribute to behaviour can help to explain

behaviours that may exist to integrate information from independent visual systems, or to maximise the quality and consistency of information given the apparatus the bird has available to it.

Chapters 2 and 3, in particular, offer a much more complete picture of the information availability problem inherent in the avian (and indeed any non-placental mammalian) brain. Much work has been undertaken to uncover what information transits the divide between the two brain hemispheres,[6-9] and these chapters are, fundamentally, a continuation of the program, further exploring this problem in opposite directions. The established understanding of birds' hemispheric integration is that visual information gathered in a conventional learning protocol with one eye and tested several hours or days later is not available with sufficient fidelity to the opposite eye to successfully complete the task at hand with only the naïve eye.[10] Chapter 2 stretches this question to the lengthier intervals of pigeon homing, positing that information may slowly be transferred across the brain divide. The rather complex results of Chapter 2 do not offer a definitive answer on this matter, but they do suggest that even lengthy periods of time do not allow a complete interhemispheric sharing of information. Pigeons flying even several weeks after first settling on a route with an initial eye still do not revert to that route with the other eye, even if some amount of relevant information is clearly available to the naïve hemisphere upon guiding its first flight.

Homing is not a solely visual task, and this may explain why monocularly experienced pigeons flying with the naïve eye are not indistinguishable from fully naïve pigeons, but these results also reinforce the key role of vision in local area homing. The case for vision, and visual landmark recognition, in pigeon homing has been building with increasingly strong evidence coming from the discovery of route recapitulation in homing,[11] but magnetoreception,[12,13] olfaction,[14] and sun compass

reckoning[15] are all components of accurate homing, and are likely more critical to navigation from unfamiliar locations than visual landmarks. However, Chapter 2 seems to leave little remaining doubt as to the importance of visual recognition in local area homing: pigeons with no other modification but that the very eye they first homed with from that site has been covered do not fly over the route they have learned. Thus beyond confirming the divide between the hemispheres as regards visual information, Chapter 2 also provides some of the strongest evidence yet for the role of visual information in pigeon homing. Birds are mostly diurnal and have high-acuity eyes and visually equipped brains, so it is not surprising to find vision critical to any given behaviour, but it reinforces this fact nonetheless; being a bird means seeing like a bird, which is to say, seeing well, and making use of it.

Chapter 3, in contrast, turns to very short learning intervals, using imprinting to enable testing of the near-instantaneous availability of information inter-hemispherically. The results from Chapter 2, along with evidence from marsh tits[1], and pigeons and chickens in Skinner boxes,[9,16] suggests that some amount of information must be available inter-hemispherically on the several-hour to several-day time scales of those experiments. Many such studies have had a lower bound of several hours for the timescales testable due to the necessity of achieving accuracy through operant or associative training with the initial eye before the naïve eye can be tested. As far as answering the question of what it is like to see like a bird, Chapter 3 brings us closer to what the naïve brain hemisphere knows immediately upon the other hemisphere (and its associated eye) "seeing it". Binary discrimination by imprinting allows the learning to be almost entirely visual, and to happen in a matter of minutes, with testing of the naïve eye occurring only 30 minutes after the very first exposure of either eye to a stimulus. The results here, unlike Chapter 2, are straightforward. Ducklings reliably revert to a chance level of preference for the learned stimulus when they switch to the

inexperienced eye. The follow up experiment reinforces this separateness of visual systems, with each eye system being able to independently maintain fidelity to opposing imprints, albeit weakly, and with neither showing dominance over the other. This implies that not only are the visual systems and their corresponding information independent of each other, but that each may be able to exert control over the bird's behaviour, even to the point that conflicting behaviours prompted by each hemisphere can nullify each other. Thus, seeing like a bird is apparently a disjointed experience, and being a bird seems tantalisingly close to having two brains.

The lateralised tool use explored in Chapter 4 is one behaviour that may have arisen to afford a bird unified information and unified action. The observation of laterality in tool holding in New Caledonian crows might be easily thought similar to the handedness seen in other tool using species,[17] but there are clear contextual differences, and Chapter 4 reveals that this behavioural laterality is, in fact, anatomically derived – arising due to the underlying laterality of the eyes. This is further contextualised by the results of the eye-capping chapters. Chapter 3, in particular, suggests that there is virtually no instantaneous visual interhemispheric transfer. This creates an information problem for monocular behaviours such as peering down a hollow whilst foraging – insofar as an individual bout of foraging does not last several hours or days (but rather, several seconds), such monocular tasks are confined to half the brain, and information which could guide aiming and manipulation of the tool gathered with one eye will never integrate with that of the other within the useful life of that information. It is thus advantageous that any given monocular task be performed consistently with the same eye (as opposed to switching back and forth), to concentrate the information gathering to one hemisphere. This being the case, even a slight superiority of one eye or hemisphere should induce the animal to the use of the same, and this is indeed what we see in the crows. This

interpretation should hold for any brain in which visual inputs remain independent. It has been pointed out that chickens focussing on a distant object will turn to direct one eye at it to the exclusion of the other.[18] To the usual explanation that the chicken is centring one of its two fovea on the target we can add that to the extent that the information gathered is immediately relevant, it is better for the chicken that only one eye (the better one) sees it. When dealing monocularly, being a bird means prioritising one eye.

More than the others, Chapter 5 involves matters of cognition, and offers evidence for a much more cognitively advanced lower bound on the capacities of *Neognathae*. Concept learning has been shown previously in crows[19] and parrots,[20] and much of this has been attributed to their relatively high intelligence and flexible learning, with respect to most other birds. The evidence presented in Chapter 5 suggests abstract concept learning in a newly hatched *Galloanserae* species, the mallard, and may imply that such abilities are taxonomically more widespread, perhaps even an ancestral characteristic of many vertebrate taxa. This would mean that being a bird, any bird, involves a greater cognitive capacity than may have been previously assumed, and it might be argued to suggest an avian mind something like that of humans, given our own abstract capacities, despite our very different brains.

More practically, the findings of Chapter 5 relate directly back to the issue raised in Chapter 2. The homing pigeons in Chapter 2 engage in a mostly visual learning to develop their recapitulated routes. Yet the pigeons, despite being incapable of flying their *exact* learned route with the naïve eye, do show some evidence of experience, differentiating them from complete naïveté. Many of the posited explanations for this come down to abstraction: perhaps though the pigeon may fail to access the visual snapshots of its landmarks, it can access the conceptual understanding of angles, bearings, sequences of manoeuvres, or sun compass

derivations associated with the landmarks gathered by the occluded eye. All of this information is non-visual or abstract, and may not be subject to the dividedness that keeps visual information in discrete halves. The ducklings monocularly imprinted in Chapter 3 are engaged in a much less abstract task than the pigeons, and this may be why the eye-switched pigeons look less like naïve birds than the eye-switched ducklings: the pigeons have concepts available throughout the unified brain on which to draw – the ducks have only their visually remembered maternal surrogate in one half of their divided brain. Concepts could be the means by which the avian brain avoids the tantalising "two-brains-in-one-head" challenge presented on first inspection of the visual system.

Conclusion

The direct conclusions of this work are simply stated: birds seemingly have little interhemispheric visual integration in the short term and incomplete integration in the long term, they may prioritize a preferred eye behaviourally to accommodate this in monocular tasks, and they make use of abstract representations from a young age. More deeply, however, it raises a number of questions, and potentially offers the way to new models for research into memory and learning, as well as a new understanding of the mental life of birds.

The avian brain is not unique in its lack of a *corpus callosum,* or other large, integrative commissure. The *corpus callosum* is a relatively young evolutionary development, arising in placental mammals about 100 million years ago, with all older lineages having no such structure.[21] Marsupials and monotremes, like birds, reptiles, and amphibians, also lack the *corpus callosum,* but instead have a massive anterior commissure – larger than that of placentals or reptiles, that seemingly accomplishes

the same role. Thus, mammals in general have all greatly enhanced their integrative capacity relative to other vertebrates.

Yet, despite the anatomical, and indeed, as this research has shown, functional separateness of the hemispheres in non-mammalian vertebrates, these animals do not fail to act as a unified whole, outside of monocular experiments. Birds and reptiles do not seem to suffer for their unintegrated hemispheres in their ordinary, binocular context. There could be a number of reasons for this: whether behavioural compensations as in the New Caledonian crows, or higher level conceptual integration made possible by abstraction of sensory information; but what remains is the question: what evolutionary pressure drove mammals to develop these large, interhemispheric pathways? Why could mammals not adopt the same solutions that seem to continue to be ample for the rest of the seeing vertebrates? One explanation holds that as mammals are the only group with topographically organized sensory maps in the telencephalon (rather than the mesencephalon, as in reptiles), they are the only lineage that required such a large forebrain commissure.[21] However, this still does not manage the evolutionarily ultimate explanation for the difference, failing to provide an adaptive reason for this sensory difference. There is fertile ground for further investigation here, especially, on the behavioural side, in marsupials, representing as they do the "third way" for integration. A suite of monocular learning tests in wallabies could be especially fruitful. Furthermore, there is some suggestion that various predatory birds may have better interhemispheric integration than other species, perhaps convergently evolved in a manner similar to that in mammals, given their visually demanding hunting.[22] A comparative approach into the problems solved by instantaneous integration would be enlightening. Such investigations could aid in pinpointing the adaptive origins of the *corpus callosum*: the presence or absence of mammalian visual integration in marsupials could date the origin of the structure, whilst a convergent

ability for instantaneous integration in raptors would point to the adaptive pressures that give rise to that ability.

Regardless of the origins of the difference between birds and mammals, the reliable performance of monocular birds in failing to transfer learned visual information could make them an ideal model system for studying memory formation. The very straightforward and largely non-invasive method of covering one eye allows for behavioural tests of something that can only be accomplished with great difficulty in mammals: isolation of half of the brain (at least within one sensory modality). Birds, though more like reptiles in their neuroanatomy, are, generally speaking, more akin to mammals in their cognitive ability, and their different brain could allow testing of complex behaviours in a conveniently malleable system.

In a sense, all the most interesting questioned raised by this work on birds are about mammals, and some of this is unavoidable. The question posed by this chapter, of what it is like to be a bird, asks that question for our mammalian perspective, and it is difficult to completely efface the assumption that our arrangement is the default. Humans only know what it is like to be a (human) mammal, and so much of what we can say about the experience of another species is to determine the ways it must be different from our own. Chapters 3 and 5 together suggest that while birds cannot integrate vision instantaneously, they can form abstractions (or *qualia*) from a very young age. Their experience of vision must therefore be different from our own, but perhaps where we integrate visually, they conceptualize both visual streams, and through that means, unify their information. This could be easily tested by combining the two experiment's methods: monocular imprinting on a relational pair, and testing on two novel pairs with the other eye. Such experiments are informative because they identify solutions to problems that cannot exist in mammals; and whilst the hard

problem of consciousness remains hard, we can only assert what it is like to be a bird by the ways it must necessarily be unlike being a human.

So then, as far as we can manage, what is it like to be a bird? To be a bird seems to mean seeing strategically – concentrating relevant information about any given situation to one eye. It seems to mean relying on non-visual information to ensure an integrated, single understanding of the world around one, and to bring unity to two, disparate visual banks of knowledge. It seems to mean being capable of basic abstraction, and possibly quite complex abstraction, with the same-different distinction only the first of a suite of investigations into concept learning that ducklings should undergo. It seems to mean being quite intelligent, or at least enough to deal with abstract concepts from a young age. It may mean, but then it may not, having a mind unlike that of any mammal, having a different or even absent sense of a unified self, but being capable of cognitive tasks and behaviours to challenge mammals despite this. At any rate, it probably means that I shouldn't eat so much duck.

References

1 Clayton, N. Lateralization and unilateral transfer of spatial memory in marsh tits. *Journal of Comparative Physiology A* **171**, 799-806 (1993).

2 Clayton, N. S. & Krebs, J. R. Lateralization and unilateral transfer of spatial memory in marsh tits: are two eyes better than one? *Journal of Comparative Physiology A* **174**, 769-773 (1994).

3 Rutz, C. & St Clair, J. J. H. The evolutionary origins and ecological context of tool use in New Caledonian crows. *Behavioural processes* **89**, 153-165 (2012).

4 Weir, A. A. S., Kenward, B., Chappell, J. & Kacelnik, A. Lateralization of tool use in New Caledonian crows (Corvus moneduloides). *Proceedings of the Royal Society of London. Series B: Biological Sciences* **271**, S344-S346 (2004).

5 Rutledge, R. & Hunt, G. R. Lateralized tool use in wild New Caledonian crows. *Animal Behaviour* **67**, 327-332 (2004).

6 Rogers, L. J., Vallortigara, G. & Andrew, R. J. *Divided brains: the biology and behaviour of brain asymmetries*. (Cambridge University Press, 2013).

7 Güntürkün, O. Lateralization of visually controlled behavior in pigeons. *Physiology & behavior* **34**, 575-577 (1985).

8 von Fersen, L. & Güntürkün, O. Visual memory lateralization in pigeons. *Neuropsychologia* **28**, 1-7 (1990).

9 Diekamp, B., Prior, H. & Güntürkün, O. Functional lateralization, interhemispheric transfer and position bias in serial reversal learning in pigeons (Columba livia). *Animal Cognition* **2**, 187-196 (1999).

10 Remy, M. & Watanabe, S. Two Eyes anc* One World: Studies of Interocular and Intraocular Transfer in Birds. *Vision, brain, and behavior in birds*, 333 (1993).

11 Biro, D., Meade, J. & Guilford, T. Familiar route loyalty implies visual pilotage in the homing pigeon. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 17440-17443 (2004).

12 Wallraff, H. G. & Foà, A. The roles of olfaction and magnetism in pigeon homing. *Naturwissenschaften* **69**, 504-505 (1982).

13 Wiltschko, W. & Wiltschko, R. Magnetic orientation and magnetoreception in birds and other animals. *Journal of Comparative Physiology A* **191**, 675-693 (2005).

14 Wallraff, H. G. Pigeon homing from unfamiliar areas: an alternative to olfactory navigation is not in sight. *Communicative & integrative biology* **7**, 6929-6943 (2014).

15 Wiltschko, R. & Wiltschko, W. The development of sun compass orientation in young homing pigeons. *Behavioral Ecology and Sociobiology* **9**, 135-141 (1981).

16 Gaston, K. E. Lack of interocular transfer of pattern discrimination learning in chicks. *Brain research* **171**, 339-343 (1979).

17 Merrell, D. J. Dominance of eye and hand. *Human Biology* (1957).

18 Dawkins, M. S. What are birds looking at? Head movements and eye use in chickens. *Animal Behaviour* **63**, 991-998 (2002).

19 Smirnova, A., Zorina, Z., Obozova, T. & Wasserman, E. Crows spontaneously exhibit analogical reasoning. *Current Biology* **25**, 256-260 (2015).

20 Pepperberg, I. M. Acquisition of the same/different concept by an African Grey parrot (Psittacus erithacus): Learning with respect to categories of color, shape, and material. *Animal Learning & Behavior* **15**, 423-432 (1987).

21 Aboitiz, F. & Montiel, J. One hundred million years of interhemispheric communication: the history of the corpus callosum. *Brazilian Journal of Medical and Biological Research* **36**, 409-420 (2003).

22 Güntürkün, O., Miceli, D. & Watanabe, M. Anatomy of the avian thalamofugal pathway. *1993) Vision, Brain and Behavior in Birds Cambridge: MIT*, 115-135 (1993).

Appendix

Published Versions of Chapters

"What's more, since the second act is a subtly different reprise of the first, he has written a play in which nothing happens, twice."

Vivian Mercier, on *Waiting for Godot*

Published Version of Chapter 2

The published typesetting of Chapter 2 begins on the following page. The citation for the chapter is as follows:

Martinho, Antone, Dora Biro, Tim Guilford, Anna Gagliardo, and Alex Kacelnik. "Asymmetric visual input and route recapitulation in homing pigeons." In *Proc. R. Soc. B*, vol. 282, no. 1816, p. 20151957. The Royal Society, 2015.

PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Martinho III A, Biro D, Guilford T, Gagliardo A, Kacelnik A. 2015 Asymmetric visual input and route recapitulation in homing pigeons. *Proc. R. Soc. B* **282**: 20151957.
http://dx.doi.org/10.1098/rspb.2015.1957

Received: 13 August 2015
Accepted: 9 September 2015

Subject Areas:
behaviour, cognition, neuroscience

Keywords:
Columba livia, homing, tecto-fugal pathway, monocular route learning, route recapitulation, visual asymmetry and learning

Authors for correspondence:
Dora Biro
e-mail: dora.biro@zoo.ox.ac.uk
Alex Kacelnik
e-mail: alex.kacelnik@zoo.ox.ac.uk

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2015.1957 or via http://rspb.royalsocietypublishing.org.

THE ROYAL SOCIETY PUBLISHING

Asymmetric visual input and route recapitulation in homing pigeons

Antone Martinho III[1], Dora Biro[1], Tim Guilford[1], Anna Gagliardo[2] and Alex Kacelnik[1]

[1]Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK
[2]Department of Biology, University of Pisa, Via Volta 6, Pisa 56126, Italy

Pigeons (*Columba livia*) display reliable homing behaviour, but their homing routes from familiar release points are individually idiosyncratic and tightly recapitulated, suggesting that learning plays a role in route establishment. In light of the fact that routes are learned, and that both ascending and descending visual pathways share visual inputs from each eye asymmetrically to the brain hemispheres, we investigated how information from each eye contributes to route establishment, and how information input is shared between left and right neural systems. Using on-board global positioning system loggers, we tested 12 pigeons' route fidelity when switching from learning a route with one eye to homing with the other, and back, in an A-B-A design. Two groups of birds, trained first with the left or first with the right eye, formed new idiosyncratic routes after switching eyes, but those that flew first with the left eye formed these routes nearer to their original routes. This confirms that vision plays a major role in homing from familiar sites and exposes a behavioural consequence of neuroanatomical asymmetry whose ontogeny is better understood than its functional significance.

1. Introduction

Birds and mammals moving in familiar landscapes acquire, process, store and use visual information, but while in the mammalian brain bilateral projections from the optic chiasma and interhemispheric connectivity through the corpus callosum ensure that visual information is readily available bilaterally, in birds visual pathways are almost completely decussated at the optic chiasm, and there is no corpus callosum. These differences make integration of visual input from the two eyes very different between these two vertebrate classes, and lead to differences in learning and memory [1]. Pigeons using visual landmarks to fly homewards from familiar locations [2] provide an opportunity to study how visual information acquired by each eye is stored and made available to contralateral visual control of navigation.

How pigeons find their way home has been subject of extensive research and lively debate for many years [3,4]. Field experiments have shown that pigeons compute their homeward bearing based on a 'map' (information on the bird's position relative to the loft, probably derived from olfactory cues when in unfamiliar territory) [2,3,5] and use a time-sensitive sun compass [6,7] or a magnetic compass [8] in assuming a homeward bearing and navigating to their loft. As they acquire experience with an area, pigeons progressively incorporate visual cues in the form of landmarks to engage in pilotage—or navigation by a series of visual landmarks—with reliance on compass information decreasing with experience [9–12]. On-board global positioning system (GPS) loggers have greatly enriched the study of pigeon homing, revealing that pigeons released repeatedly from the same site form individually idiosyncratic routes that are tightly recapitulated on subsequent flights [10,11,13]. Furthermore, their routes often incorporate long linear landmarks such as roads, railway lines and rivers [9].

The pigeon visual system is lateralized at both neuroanatomical and functional level, as shown in birds tested in laboratory cognitive tasks [14].

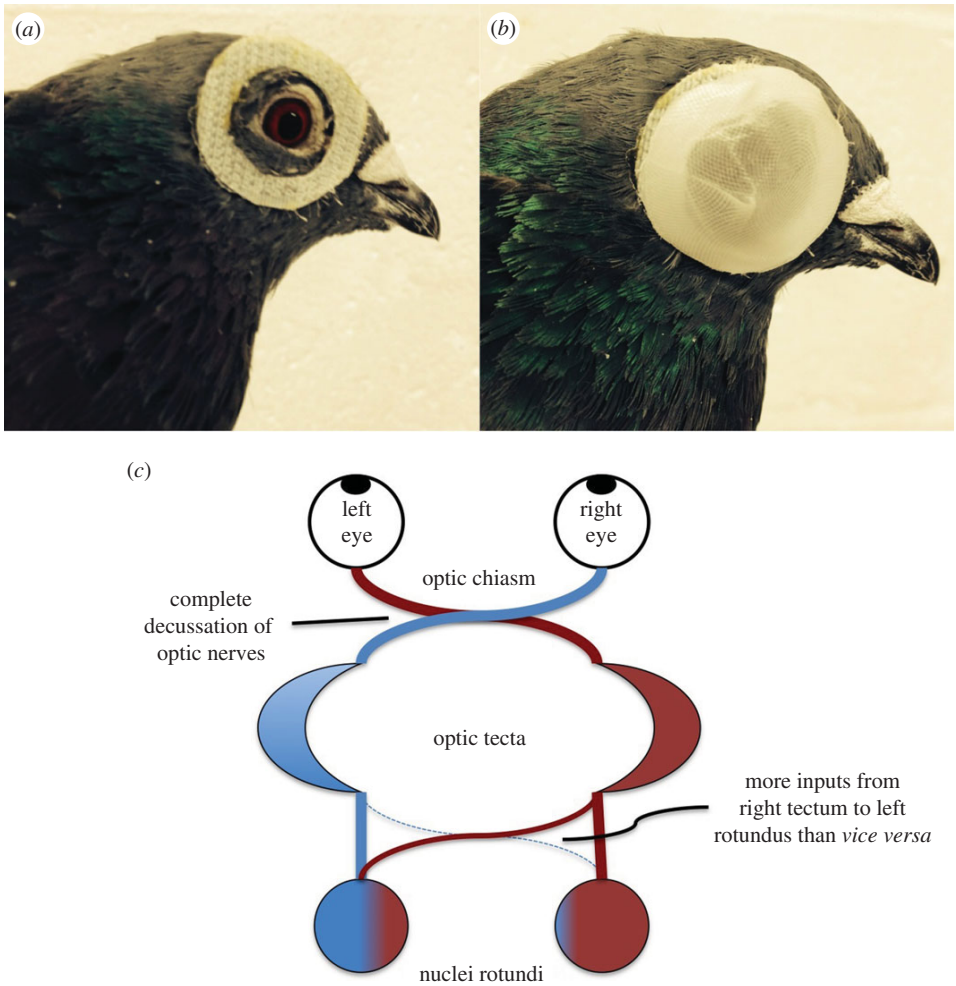


Figure 1. Pigeon with eye ring and eye cap, and generalized structure of pigeon tecto-fugal system. (*a*) Each pigeon was fitted with two eye rings as shown. The 'hook' side of Velcro was used for the ring attached to the pigeons' feathers as its thinner profile ensured forward vision would not be compromised. (*b*) The eye caps were constructed of a double layer of flexible etched plastic. The seam in the eye cap was consistently oriented posteriorly to ensure a homogeneous image in the forward direction. (*c*) Schematic of the pigeon tecto-fugal visual system, detailing the asymmetry of inputs. Adapted from [23].

Functional asymmetries have also been found for spatial tasks involving memorization and processing of cues in both laboratory settings and natural behaviours, such as homing [15]. In particular, the functional contribution of the left and right side of the brain in homing behaviour has been the subject of experiments focusing on the hippocampal formation and olfactory and visual systems [15,16]. Although both the left and right parts of the hippocampal formation seem to be involved in familiar landmark-based navigation [17], an intact left hippocampus is needed for olfactory map learning in young pigeons raised confined [18], probably due to the critical role of the left hippocampus in sun compass-mediated spatial learning [19] through which the association of wind-born odours with wind direction is memorized. In the olfactory system, an asymmetric involvement in favour of the left piriform cortex and of the right nostril has been highlighted [15].

Within the visual system, pigeons' optic nerves completely decussate at the optic chiasm, projecting exclusively to the tectum contralateral to the input eye [20], meaning that direct visual inputs to a hemisphere can be eliminated by capping an eye. The ascending tecto-fugal and thalamo-fugal visual pathways do allow for indirect visual input to the contralateral hemisphere [21], but while each tectum projects to the ipsilateral diencephalic nucleus rotundus roughly equally, more projections from the right tectum are shared contralaterally to the left nucleus rotundus and entopallium than from left to right [1,14,22]. This asymmetry is developmentally related to the orientation of the pigeon embryo, whereby the right eye is exposed to more light during incubation [14,23]. The importance of this light exposure to interhemispheric integration has been shown in visual transitive inference tasks performed by monocularly occluded pigeons [20]. Memory formation and storage may be lateralized, with predominance of the left hemisphere [24], and further asymmetries of projections have been found in both the ascending and descending visual systems [25]. This fits into the wider model of the avian visual system as being lateralized differently for specific tasks and roles [26]. Nonetheless, this model is largely derived from chickens, and it is likely that detailed patterns of lateralization differ between pigeons, chickens and other birds.

Here, we investigate whether the asymmetries of the visual memory system are reflected in pigeons' route fidelity when switching from monocular homing with an 'experienced' to a 'naive' eye. We trained 12 pigeons to fly home under reversible, non-invasive monocular occlusion (figure 1) for 18 flights before subsequently flying another 18 flights from the same release point with the opposite eye blocked. This was followed by five flights with the original eye blocked, and finally by five

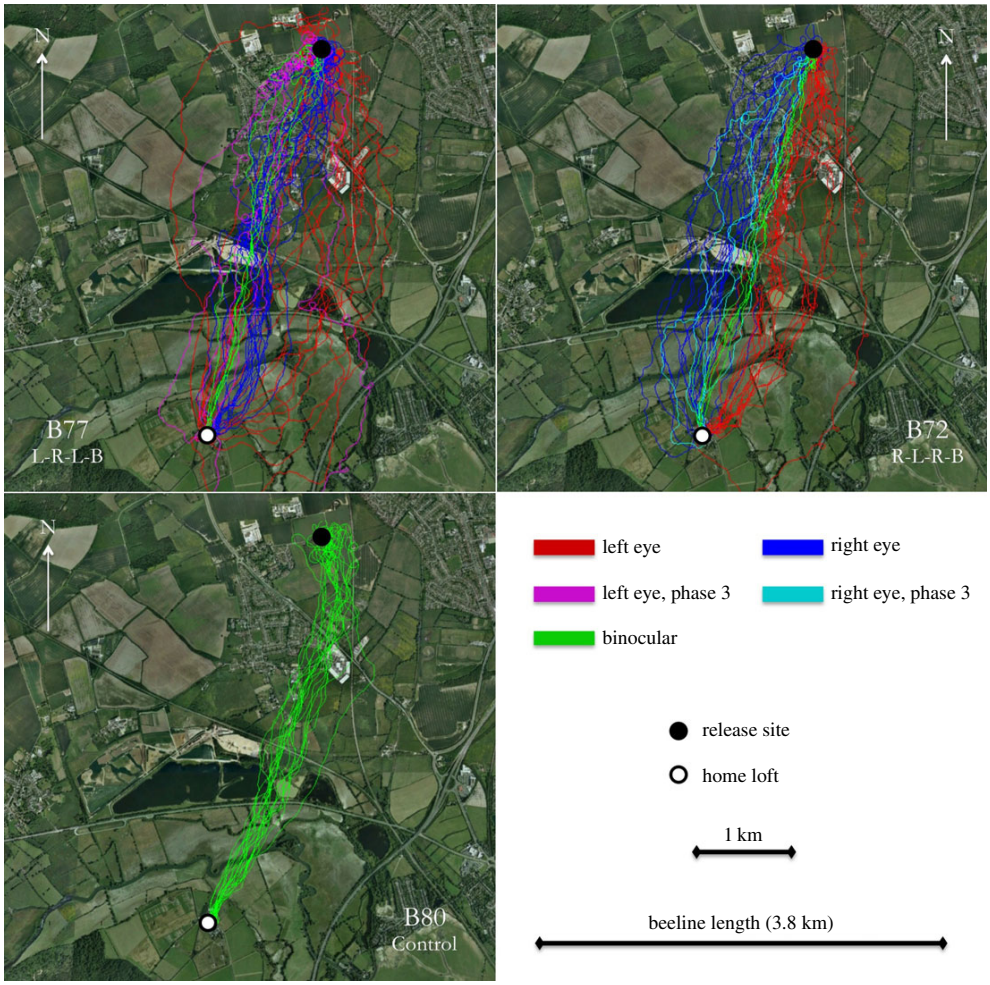


Figure 2. Examples of flight series for two experimental and one control pigeon. Each experimental pigeon flew 46 flights in either a '18 left, 18 right, 5 left, 5 binocular' or '18 right, 18 left, 5 right, 5 binocular' pattern (referring to the eye open, not the eye occluded). Bird IDs and treatment received are indicated in the bottom right corner of each panel. Left-eyed flights are shown in red (magenta for phase 3, bird B77 only), whereas right-eyed flights are blue (cyan for phase 3, bird B72 only). Binocular flights are shown in green. The control pigeons flew 18 flights each, all of which were binocular.

binocular flights, completing a total of 46 flights per bird, in four phases. Our results reflect a poverty of interhemispheric exchange of visual homing information and a hemispheric imbalance of information availability in the pigeon brain.

2. Results

The 12 experimental birds flew in two groups defined by the sequence of which eye was available (i.e. the eye which was not occluded) in each phase of experimentation: 'left–right–left–binocular' (hereafter LRLB) and 'right–left–right–binocular' (hereafter RLRB). A control group of four birds flew 18 non-occluded flights. Positional fixes recorded at 5 Hz by on-board GPS loggers allowed us to reconstruct the pigeons' flights (see 'Experimental procedures') and to compare paths flown. The set of the first 18 flights for each group is referred to as 'phase 1' and the second 18 flights, flown with the other eye, as 'phase 2'. The subsequent five flights, flown with the original eye, comprise 'phase 3'. For clarity, except where otherwise indicated, the descriptors 'left eye' and 'right eye' refer to the eye that is open (not occluded) for the flight being described.

Visual inspection of the trajectories revealed consistent patterns, as displayed by exemplars in figure 2. For all experimental birds, the first several flights show properties distinct from subsequent monocular flights and from all control flights. In these early monocular flights, the pigeons' routes deviate from the beeline between the release site and the loft in the direction of the available eye. As the pigeons become more experienced with a given eye, the routes migrate towards the beeline. Furthermore, during early flights, the pigeons often perform many tight loops around the open eye—clockwise when the right eye is open and counterclockwise when the left eye is open—possibly attempting to gather information from a 360° field of view. This is a behaviour not previously described, as far we are aware. At no point in the study was a bird seen to loop around an occluded eye. Control birds did perform loops, but as both eyes were open, flights included loops in both directions. We quantified each bird's looping rate as loops per kilometre of route flown, excluding loops made around the release site and home loft (figure 3). Among experimental birds, looping rate begins high and subsides through subsequent flights, before returning to a high rate when the occluded eye is switched, and subsiding in a similar pattern. We compared the looping rate across flights within each of the controls, and the first two phases of each of the LRLB and RLRB birds, using a two-way repeated measures ANOVA. The 18 flight phases of the

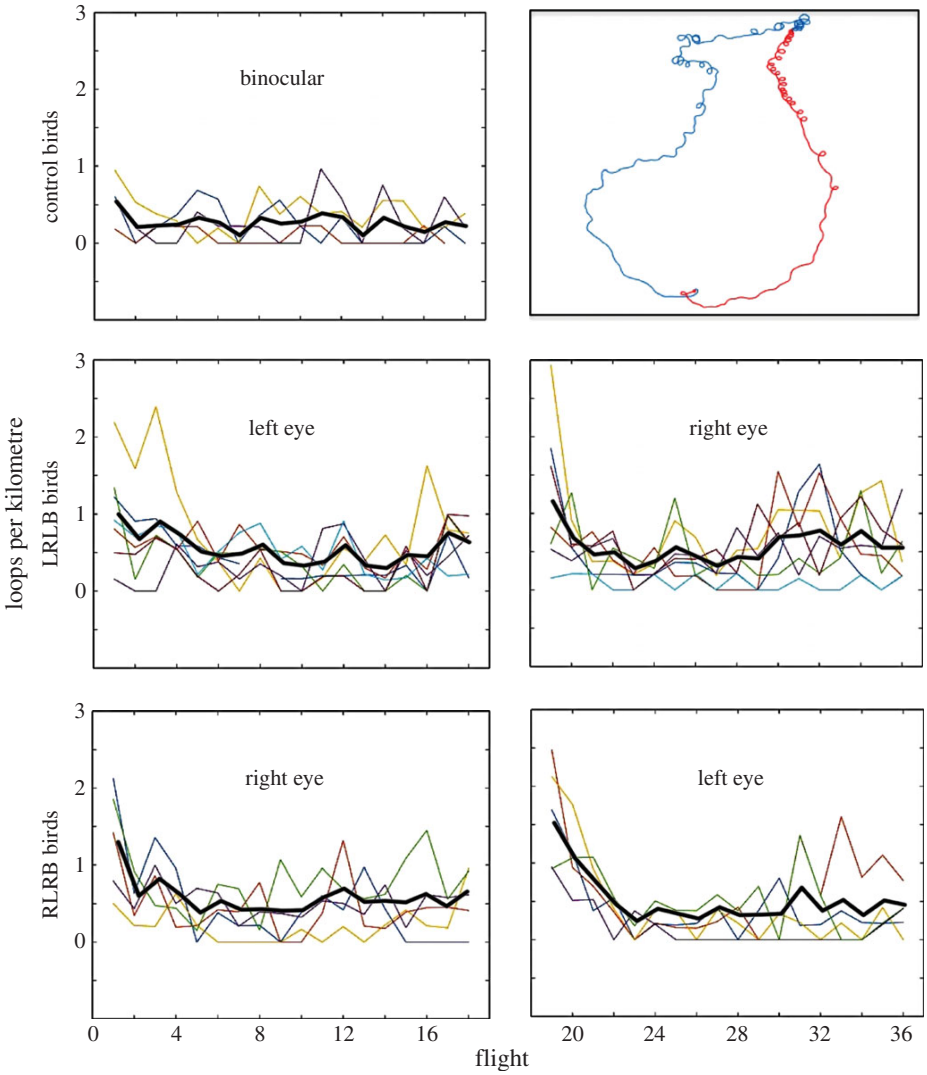


Figure 3. Looping rate analysis. Monocularly occluded birds perform tight loops (approx. 25–50 m in diameter) around the open eye during early flights as seen in the panel at top right (the blue track is a right-eyed bird, the red, left). The binocular controls and both phases of experimental flights show significant decrease in looping rate throughout the 18 flights. The heavy black lines show the mean value across pigeons in the group for each flight. High early looping rates may reflect information gathering by visually hemicompromised pigeons.

experimental birds were considered separately, such that the ANOVA included five groups of 18 repeated measures (flights). The rate of looping differs significantly across flights ($F_{17,340} = 7.162, p < 0.001$), indicating that reduced looping rates correlate with increased experience homing over a given route. There was no significant interaction between looping rate and group, indicating that this effect is similar across groups. Both experimental groups showed an increase in looping rate following the first eye switch followed by a decline with experience. The cause for this looping may simply be that it takes time to adjust to the new circumstances, or, more interestingly, that using an eye that is naive for the release site, in the absence of interocular transfer, requires learning of new visual landmarks, and that looping in the direction of the naive eye reflects this search for visuo-spatial input. One possible mechanism in either case may be that under binocular conditions, the view of each eye 'pulls' the animal to the corresponding side, and being newly deprived of one eye's counterbalance results in a loop, until this is corrected by experience.

To test whether information input to the contralateral hemisphere was more effective in one direction than the other, we assessed how the birds' trajectories changed across phase changes. To do this, we used a distance-to-beeline analysis (figure 4). Each flight was sampled at points representing GPS positional fixes collected at 5 Hz. This analysis computes the mean perpendicular distance in metres between the beeline and each point in a given flight, assigning positive values to scores left of the beeline and negative values to those to the right, with respect to the direction of flight. Figure 4*a* shows the beeline analysis for each flight. To compare the degree to which routes changed across phases, we computed the difference in distance to beeline between each bird's first flight after an eye switch and the mean of the 10 flights before the switch (figure 4*b*), for both the initial switch from phase 1 to phase 2 (i.e. switching to a 'naive' eye) and the subsequent switch from phase 2 to phase 3 (i.e. switching back to the eye that became 'experienced' in phase 1). We calculated the magnitude of the change from pre-switch mean to the first post-switch flight for each bird and compared the groups via two-sample *t*-tests, as shown in figure 4*b*. The average ($\pm$s.e.) signed magnitude of the changes in distance to the beeline after the first (naive) switch was -250.2 ± 67.0 m for the

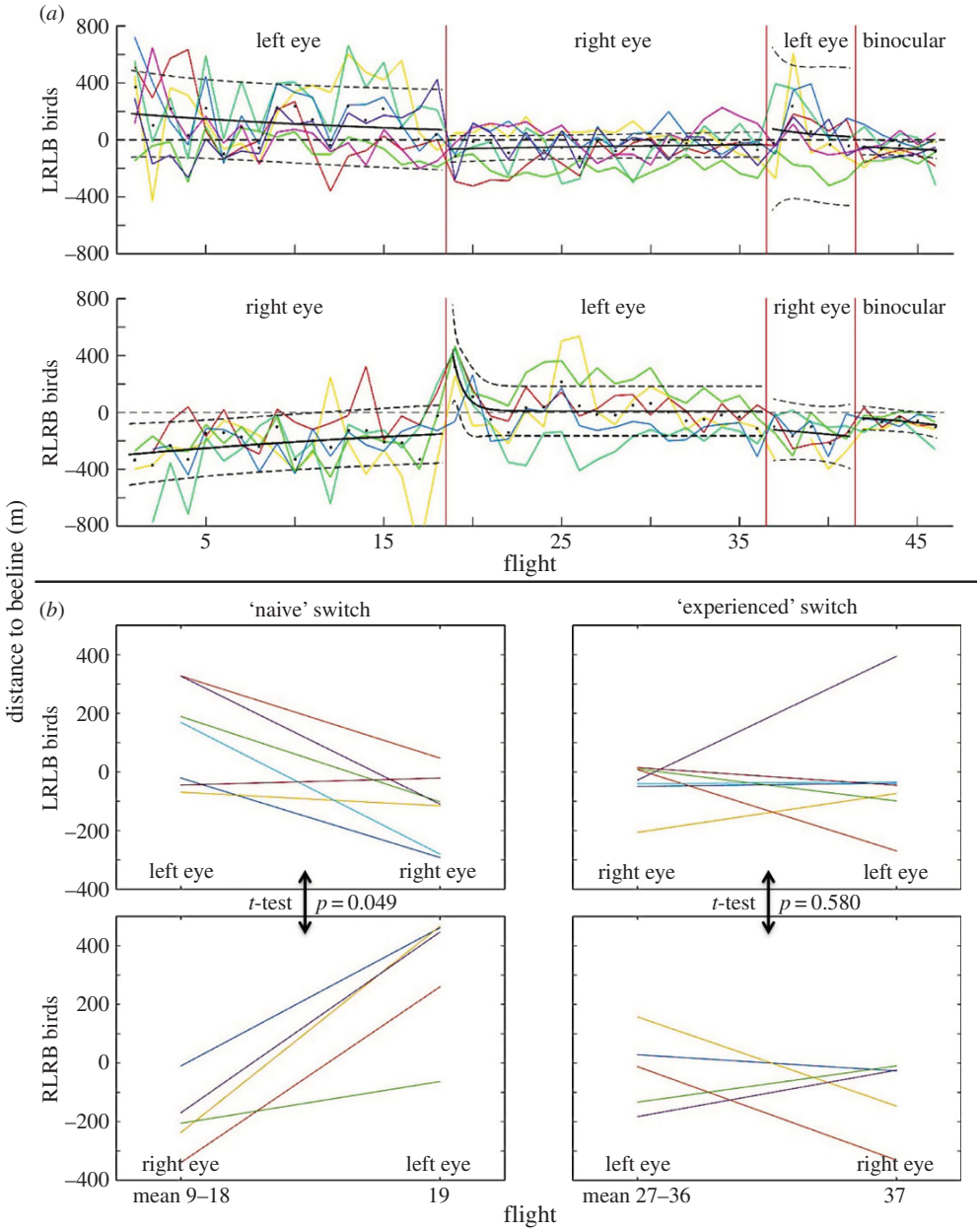


Figure 4. Beeline analysis. The beeline analysis calculates the distance to the beeline for each point along a flight and gives the mean of these distances as the score for that flight. (*a*) Each bird's score is shown for each flight. The black lines show a fitted exponential curve with 95% confidence intervals (fitted parameters are given in the electronic supplementary material). The 'left eye' and 'right eye' labels refer to the open eye. Points to the right of the beeline, with reference to the direction of motion, are scored as negative values, while those to the left are positive. (*b*) For each of the two eye-switches from phase 1 to phase 2 and phase 2 to phase 3, the mean of the final 10 flights before the switch is compared with the first flight after the switch.

LRLB group and 506.9 ± 98.5 m for the RLRB group. In addition to the obvious sign difference (figure 4*b*), the absolute value of the difference between the first and second phase for the RLRB group was significantly greater than for the LRLB group ($p = 0.049$, two-sample *t*-test), corresponding with the move across the beeline seen in the RLRB graph, indicating that the asymmetric effect of the treatment was true at individual level and not an artefact of averaging. This implies that the LRLB group's route was less affected by the eye switch than was the case for the RLRB group. It is possible that information concerning a general spatial affinity for the landmarks acquired with the left eye/right hemisphere system (LRLB group) reached the left hemisphere via an indirect input to a greater degree than in the opposite direction (RLRB group). There was no significant difference between groups in the absolute value of change between the second and third phases ($p = 0.580$).

While the LRLB birds did fly nearer to their phase 1 routes in phase 2 than was the case for RLRB birds, the beeline analysis does not determine the fidelity of route recapitulation (similar mean distance to the beeline can result from different trajectories). For this reason, route fidelity was examined using a 'nearest neighbour analysis' (figure 5*a*), in which each flight within a bird's series was compared to the immediately preceding flight. The details of this analysis are presented in the 'Experimental procedures' section below. The nearest neighbour analysis shows how the route differs between consecutive pairs of flights [27], providing a measure of

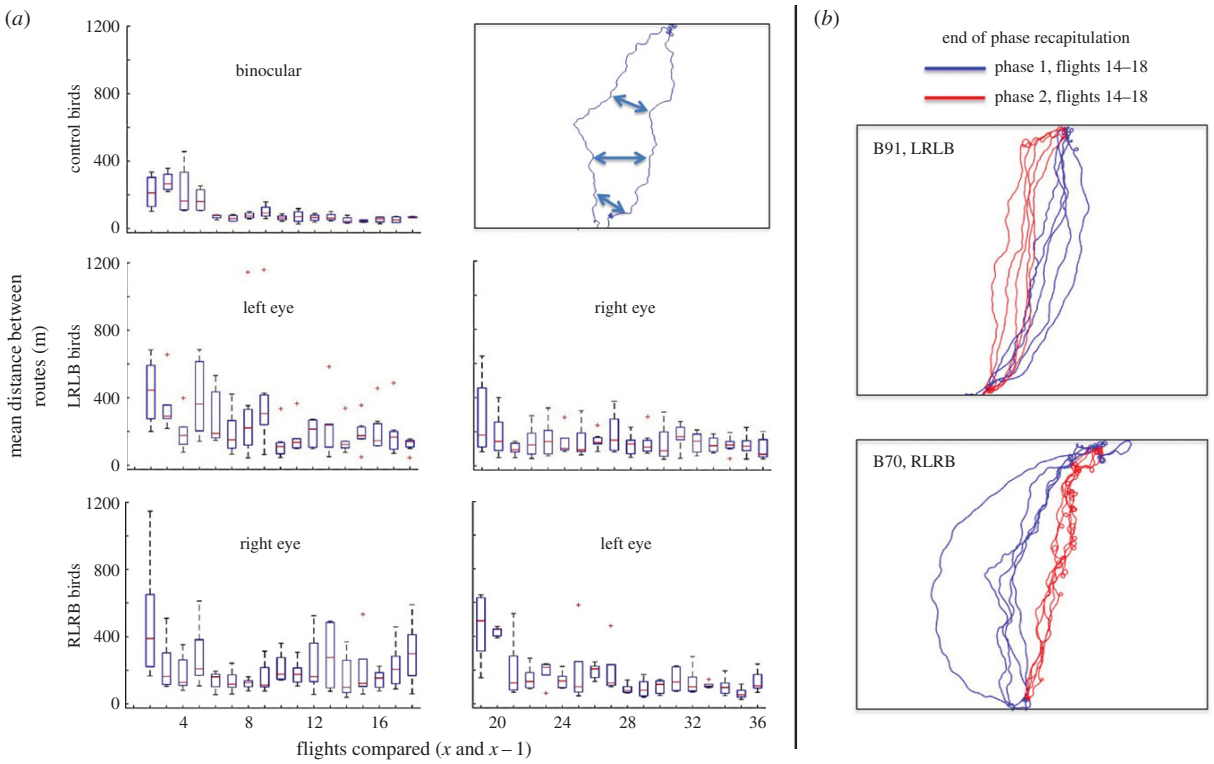


Figure 5. Nearest neighbour analysis of route development. (*a*) The nearest neighbour analysis calculates the degree to which a flight has changed with respect to the previous flight, as seen in the panel at top right (see 'Experimental procedures' for details). The blue arrows represent the shortest distances between the two flights, computed at each point in the flight (here, three are shown for clarity). The pairwise comparisons of flights in each group (controls, LRLB and RLRB) are shown as boxplots about the median. The 'left eye' and 'right eye' labels refer to the open eye. (Note that since there is no flight previous to the first, the first frame is left empty.) Recapitulation occurs when nearest neighbour values remain low, while adjacent high values indicate variability of route. (*b*) Examples showing that both LRLB and RLRB birds recapitulated over a different route in each phase.

variability or fidelity of a bird's recapitulation of its route. Figure 5*a* shows the nearest neighbour results for the controls and the first phases of the two experimental groups. The controls show a rapid progression from early variability to asymptotic fidelity. These results were compared to the first phase of both experimental groups via two-way repeated-measures ANOVAs. All three groups showed a significant main effect of decrease in nearest neighbour distance with experience across the 17 pairwise comparisons ($F_{16,176} = 1.827$, $p = 0.031$), but no significant difference between the groups ($F_{32,176} = 0.876$, $p = 0.662$). The phase 2 results of the experimental groups were compared in the same way, with both groups again showing a significant decrease across the 18 pairwise comparisons (including the cross-phase comparison of the last flight in phase 1 to the first in phase 2; $F_{17,170} = 7.098$, $p < 0.001$), and a significant difference in nearest neighbour values between the two groups ($F_{17,170} = 2.167$, $p = 0.007$). This is consistent with the results of the beeline analysis, which showed that the RLRB group displayed a larger shift in spatial location of route across the eye switch. Both groups were affected by the switch from the first eye to the second—the high nearest neighbour values imply that the first flight of phase 2 did not recapitulate the last flight of phase 1 in either group. This is consistent with the findings of the beeline analysis, which showed that both groups' routes changed, albeit to different degrees, across the initial eye switch. This implies that while both groups achieved consistent recapitulation by the end of each of phases 1 and 2, both groups also modified their routes after the switch, as nearest neighbour distances increased at the beginning of phase 2.

The high nearest neighbour values at the beginning of phase 2 do not exclude the possibility that the birds produced high variability as a result of the eye switch, but then settled back to the same recapitulated route as before the switch—especially the LRLB birds, which were shown in the beeline analysis to achieve similar scores across phases. However, as we noted earlier, similarity of beeline score does not imply route recapitulation, and a visual inspection of the final five flights for each bird in each of phases 1 and 2 reveals that this was not the case. Figure 5*b* shows representative examples of these flights for each group (equivalent figures for all birds are supplied in electronic supplementary material, figure S1). As can be seen, while within phase flights are near to each other, routes differ across phases. Some birds, especially in the LRLB group, did fly very near their previous route, but the shape of their route differed between the two phases. While the LRLB birds did show slightly higher route fidelity after the eye switch than the RLRB birds, the complete information necessary for precise recapitulation, perhaps including sequence, bearings or other onward guidance connecting one landmark to another, was not sufficiently available to either group to allow strict recapitulation.

3. Discussion

Our results indicate that in pigeons input of visual homing information from each eye is not closely integrated with input from the other eye, that the level of integration is asymmetric and that this asymmetry has functional consequences.

Pigeons' routes learned solely with one eye are not recapitulated when flying with the other, naive eye, and this failure to recapitulate occurs regardless of which eye learns first, though birds that home first monocularly with the left eye fly significantly nearer to their left-eyed route when subsequently flying right-eyed than vice versa. This suggests that the various commissures in the pigeon visual pathway allow insufficient contralateral input of route information for strict recapitulation using the naive eye. However, route recapitulation by visual landmarks requires both visual and non-visual memory, as a pigeon must both recognize each landmark visually and remember which direction to take and which landmark comes next in sequence at each point to follow its established route tightly. It is thus possible that though this strict sequence was lost in both groups, the LRLB group's more general visual recognition and attraction to the previously learned landmarks may have endured across phases and may reflect the asymmetry of the pigeon's visual pathway, especially in the ascending tecto-fugal pathway.

Concordant with the asymmetry present in the commissures of the tecto-fugal visual pathway, birds flying with the right eye after learning initially with the left eye, as seen in the LRLB group, fly significantly nearer to their original (left eye), first-learned route than birds flying in the opposite order. Thus, although neither group using the second eye followed the precise set and sequence of visual landmarks learned with the first eye, the asymmetry of the pigeon visual system [1,14,23] means that information acquired with the left eye is more readily available to control visually guided behaviour by the other eye than the opposite. Even after repeated experience, the phase 2 routes of the RLRB group settle farther from their initial (left-eyed acquired) routes than those of the LRLB birds, possibly because the weaker projections from the left optic tectum to the right nucleus rotundus are much reduced relative to the opposite side [1,14,23], though other neural asymmetries may contribute to this difference [25]. Thus, though neither eye can access the exact sequence of landmarks determining the route established with the other eye, birds flying right-eyed after left fly more closely to the routes learned with the left eye. The fact that no significant change in route was found in either group upon returning to the first eye in the third phase is consistent with previous observations that pigeons retain learned recapitulated routes over long periods of time. In both groups, by the beginning of the third phase, 18 flights had been flown by each eye, thus allowing whichever eye was used in the third phase to access those visual memories and home accordingly.

Our beeline analysis results further indicate that the difference in performance cannot be solely attributed to lateralization—the superiority of one eye or hemisphere over the other for homing performance. If this were the case, we would expect to find similar performance for a given eye across both groups in all phases. Instead, we see that homing performance for a given eye varies depending on the sequence of training and previous experience. This suggests that our results derive from the asymmetry of visual inputs to the two hemispheres, rather than simple superiority of one hemisphere or eye.

These results confirm the suspected effects of neuroanatomical division and asymmetry of visual processing, and contribute to our understanding of visual lateralization in homing for pigeons with some homing experience. A previous study on monocular homing inferred the importance of the eyes for homing lateralization from different homing speed and vanishing bearings with each eye, but argued that since both naive and experienced birds showed vanishing bearing deflection and lateralization of performance, this was not an effect of visual memory but potentially of lateral specialization for magneto-reception or optic flow [28]. However, naive and experienced pigeons navigate by different strategies; namely, naive pigeons rely mostly on site-specific compass orientation, whereas experienced pigeons transition to pilotage-based orientation within the familiar area [29]. By using GPS technology that allows investigation of the fidelity of homing to previously recapitulated routes, we have shown that the better performance of the LRLB pigeons in phase 2 (when flying with the previously naive right eye) is likely to be a result of the left hemisphere's more complete visual memory of homing routes. The phase 2 results indicate that pigeons experienced with both eyes separately show right eye/left hemisphere superiority in homing, a pattern previously noted in pigeons flying monocularly after simultaneous binocular experience [30], and in pigeons navigating in an indoor arena strewn with visual landmarks [31].

Finally, our results reaffirm the importance of vision itself in the process of pigeon homing. At one time the subject of debate, the degree of relevance of visual information in allowing pigeons to reliably home remains in question. While the formation of, and loyalty to, individually idiosyncratic homing routes have been taken as key support for the importance of visual information and memory in familiar area homing [9], these inferences were indirect. The current study is the first to assess route recapitulation while manipulating visual information input directly during homing, and demonstrates that monocular homing performance follows well the pattern established in other purely visual tasks. Pigeons undertaking a monocular binary discrimination task after binocular training show better performance with the right eye than with the left, again probably a consequence of the right eye's access to a more complete visual memory [32]. Our results demonstrate a similar pattern for familiar area homing, and thus situate this homing within the pigeon's visually controlled behaviours.

4. Experimental procedures

(a) Subjects and materials

Sixteen homing pigeons 2 years of age and of both sexes were used. Pigeons were housed at the Oxford University Field Station, Wytham, UK, where they had also been bred. Pigeons were kept at free-feeding weight with unlimited access to water, grit and mixed grain food, and allowed to fly freely from the loft daily.

Twelve experimental birds were prepared for attachment of GPS logging devices by trimming non-flight feathers on their backs and applying a 4 cm strip of Velcro with fast-drying glue. All GPS devices were initially attached via the Velcro method, but in cases in which the strip became loose during the course of the experiment, the attachment method was switched to the use of an elasticated harness (for further details, see 'Irregularities' below). Rings of Velcro to facilitate eye cap attachment were glued around each eye as in figure 1. QStarz BT-Q1300ST 5 Hz personal GPS loggers were modified for attachment to pigeons by removing the outer plastic covering. Eye caps (figure 1) consisted of a 2 cm diameter disc composed of a double layer of etched plastic to allow for light penetration

but prevent shape identification. The remaining four birds formed a control group and were prepared in the same fashion for GPS attachment, but were not fitted with eye rings.

All pigeons received basic training of at least three flock releases and three solo releases from locations roughly aligned with the four cardinal directions, approximately 2 km from the loft, alternated to prevent directional bias. All training flights were flown binocularly.

(b) Releases

Releases investigated homing in birds completely naive to the release site. All birds' experimental releases were solo flights and birds were released 7 min apart to prevent *ad hoc* flock formation. The pigeons had no previous homing experience beyond basic training. A single release point was used, but all analyses compared changes in route within subject and between flights, rather than location of route with reference to the landscape. Any bias towards landmarks from this release site is therefore unlikely to have affected our results. Furthermore, the use of a single release site minimized loss of birds.

(i) Experimental groups

The 12 experimental birds were divided into two groups, one of seven (LRLB group) and one of five (RLRB group). The groups initially contained eight birds each, but one bird from the LRLB group and three birds from the RLRB group failed to home during the first flight (see 'Losses and Irregularities' below).

The pigeons were released from a novel location 3.8 km from the loft. The releases comprised 50 flights in four phases over the course of three months.

Phase 1 (flights 1–20) consisted of first-eye training and testing. The LRLB group flew with their left eye (right eye covered) and the RLRB group with the right eye (left eye covered) during all flights in phase 1. The first 18 of the 20 phase 1 flights completed by each bird were included in analysis. The final two flights were reserves, included to accommodate occasions when a bird missed a flight for one of a number of reasons or a GPS logger failed (see 'Irregularities' below). In cases in which a bird did not miss two flights within the set of 20, any flights beyond the 18th (one to two flights) were excluded from consideration.

Phase 2 (flights 21–40) consisted of second-eye training and testing, and followed the same pattern as phase 1. In phase 2, the LRLB group flew with their right eye (left covered) and the RLRB group with their left eye (right covered). As in phase 1, the pigeons flew 20 flights, of which 18 were included in analysis, and two were reserves.

Phase 3 (flights 41–45) consisted of re-testing the first eye. The LRLB group flew with their left eye and the RLRB group with their right eye. All pigeons flew five flights in phase 3.

Phase 4 (flights 46–50) consisted of binocular testing. All 12 pigeons flew five flights with both eyes uncovered.

(ii) Control group

The four control birds were released binocularly 18 times from the same site as the experimental birds, following the same protocols. The first 13 flights comprised training and the last five flights (flights 14–18) were used to determine 'normal high-familiarity homing' for comparison with experimental birds. The control birds did not experience any irregularities.

(c) Data processing

GPS traces of flight paths were converted into comma-separated-values format by proprietary software included with the GPS loggers. All subsequent processing and analysis of flight paths was performed in MATLAB. All flights were trimmed for speed (retaining all points for which speed was greater than $0.5\ m\ s^{-1}$ for 30 s before or after) to exclude waiting time before release and after re-entering the loft. Flights were then trimmed to exclude points within 200 m of the start and end-points to exclude time when all flights are constrained to be very close together. To assist comparisons with other experiments, a table of commonly used pigeon homing metrics before the advent of GPS location may be found in the electronic supplementary material.

(i) Nearest neighbour analysis

The nearest neighbour analysis holds one of the two flights compared in reference, and for each point along the reference measures the distance to the closest point on the compared flight in any direction (figure 5*a*, inset). For each pairwise comparison, both flights are held in reference and compared to the other in turn, and the mean of these two series of distances provides the mean nearest neighbour distance between the two flights.

(d) Losses and irregularities

(i) Losses

The first flight of the experimental releases was performed with a slightly modified version of the eye cap that incorporated a layer of tracing paper to partially occlude light. Four of 16 birds failed to home during this first flight. As a result of this high failure rate, the eye caps were restructured to exclude the tracing paper and include a second layer of etched plastic, preserving the shape disruption but allowing for still more light penetration. Following this modification, the remaining 12 birds homed successfully. Among the 12 birds that successfully homed during the first flight, performance was reduced, with long routes very far from the beeline.

(ii) Irregularities

On some occasions, a bird did not produce data during a given flight. When this occurred, a reserve flight was used. All such irregularities occurred during phases 1 and 2, and in no case did a bird require more than two reserve flights. Causes of irregularities included sudden weather changes, GPS device failure and birds not yet returned from free flight around the loft. Additionally, nine birds' Velcro strips fell off during the course of the experiment. As this was a result of feather moulting beneath the strip, the strip could not be reattached until the following season, and so was replaced by a 'backpack' consisting of a fabric pouch to hold the GPS logger, attached to the pigeon by elasticated straps that go around the wings and across the keel. In seven of these cases, the bird in question was mounted with the backpack and excluded from flights for the remainder of the day to allow it to become accustomed to the backpack. Each of these birds wore the backpack for the remaining duration of the experiment and during all remaining flights.

Ethics. All experiments were conducted with the approval of the University of Oxford's Zoology Animal Welfare Ethical Review Board (ref. APA/1/5/Zoo; 7 November 2013).

Data accessibility. GPS tracks of all flights in the experiment are available on Dryad: http://dx.doi.org/10.5061/dryad.2c47d.

Authors' contributions. The experiment was devised by A.M. with input and advice from D.B., T.G., A.G. and A.K. Birds were prepared for experiments by A.M., with assistance from A.K. and D.B. Pigeon releases were carried out by A.M. Data processing was carried out by A.M., and analysis was performed by A.M. with input and assistance from D.B., T.G., A.G. and A.K. A.M. wrote the manuscript with editing and assistance from D.B., T.G., A.G. and A.K.

Competing interests. We declare we have no competing interests.

Funding. D.B. was supported by a Royal Society University Fellowship.

Acknowledgements. We thank Isobel Watts, Lucy Taylor and Takao Sasaki for their cooperation in the shared use of the pigeon colony, and Mateo Lewis and Emma Truswell for assistance with pigeon releases. We are grateful to Lucy Larkman and David Wilson for their technical support and help with animal husbandry. We thank Benjamin Pettit for instructing A.M. on the processing of pigeon flight data in MATLAB, and Richard Mann and Robin Freeman for writing much of the code used in this processing.

References

1. Rogers LJ, Vallortigara G, Andrew RJ. 2013 *Divided brains: the biology and behaviour of brain asymmetries*. Cambridge, UK: Cambridge University Press.
2. Holland RA. 2003 The role of visual landmarks in the avian familiar area map. *J. Exp. Biol.* **206**, 1773–1778. (doi:10.1242/jeb.00365)
3. Wallraff HG. 2014 Pigeon homing from unfamiliar areas: an alternative to olfactory navigation is not in sight. *Commun. Integr. Biol.* **7**, 6929–6943. (doi:10.4161/cib.28565)
4. Wiltschko R, Wiltschko W. 2003 Avian navigation: from historical to modern concepts. *Anim. Behav.* **65**, 257–272. (doi:10.1006/anbe.2003.2054)
5. Gagliardo A. 2013 Forty years of olfactory navigation in birds. *J. Exp. Biol.* **216**, 2165–2171. (doi:10.1242/jeb.070250)
6. Schmidt-Koenig K. 1990 The sun compass. *Experientia* **46**, 336–342. (doi:10.1007/BF01952166)
7. Guilford T, Taylor GK. 2014 The sun compass revisited. *Anim. Behav.* **97**, 135–143. (doi:10.1016/j.anbehav.2014.09.005)
8. Wiltschko W, Wiltschko R. 2005 Magnetic orientation and magnetoreception in birds and other animals. *J. Comp. Physiol. A* **191**, 675–693. (doi:10.1007/s00359-005-0627-7)
9. Guilford T, Biro D. 2014 Route following and the pigeon's familiar area map. *J. Exp. Biol.* **217**, 169–179. (doi:10.1242/jeb.092908)
10. Biro D, Meade J, Guilford T. 2004 Familiar route loyalty implies visual pilotage in the homing pigeon. *Proc. Natl Acad. Sci. USA* **101**, 17 440–17 443. (doi:10.1073/pnas.0406984101)
11. Biro D, Meade J, Guilford T. 2006 Route recapitulation and route loyalty in homing pigeons: pilotage from 25 km? *J. Navig.* **59**, 43–53. (doi:10.1017/S0373463305003541)
12. Gagliardo A, Ioalé P, Bingman VP. 1999 Homing in pigeons: the role of the hippocampal formation in the representation of landmarks used for navigation. *J. Neurosci.* **19**, 311–315.
13. Meade J, Biro D, Guilford T. 2005 Homing pigeons develop local route stereotypy. *Proc. R. Soc. B* **272**, 17–23. (doi:10.1098/rspb.2004.2873)
14. Güntürkün O, Stüttgen MC, Manns M. 2014 Pigeons as a model species for cognitive neuroscience. *e-Neuroforum* **5**, 86–92. (doi:10.1007/s13295-014-0057-5)
15. Prior H. 2006 Lateralization of spatial orientation in birds. In *Behavioural and morphological asymmetries in vertebrates* (eds Y Malashichev, AW Deckel), pp. 75–85. Washington, DC: Landes-Bioscience.
16. Pecchia T, Gagliardo A, Filannino C, Ioalè P, Vallortigara G. 2013 Navigating through an asymmetrical brain: lateralisation and homing in pigeon. In *Behavioral lateralization in vertebrates* (eds D Csermely, L Regolin), pp. 107–124. Heidelberg, Germany: Springer.
17. Gagliardo A, Odetti F, Ioalè P, Bingman VP, Tuttle S, Vallortigara G. 2002 Bilateral participation of the hippocampus in familiar landmark navigation by homing pigeons. *Behav. Brain Res.* **136**, 201–209. (doi:10.1016/S0166-4328(02)00125-0)
18. Gagliardo A, Ioalè P, Odetti F, Bingman VP, Siegel JJ, Vallortigara G. 2001 Hippocampus and homing in pigeons: left and right hemispheric differences in navigational map learning. *Eur. J. Neurosci.* **13**, 1617–1624. (doi:10.1046/j.0953-816x.2001.01522.x)
19. Gagliardo A, Vallortigara G, Nardi D, Bingman VP. 2005 A lateralized avian hippocampus: preferential role of the left hippocampal formation in homing pigeon sun compass-based spatial learning. *Eur. J. Neurosci.* **22**, 2549–2559. (doi:10.1111/j.1460-9568.2005.04444.x)
20. Manns M, Römling J. 2012 The impact of asymmetrical light input on cerebral hemispheric specialization and interhemispheric cooperation. *Nat. Commun.* **3**, 696. (doi:10.1038/ncomms1699)
21. Karten HJ, Hodos W. 1970 Telencephalic projections of the nucleus rotundus in the pigeon (*Columba livia*). *J. Comp. Neurol.* **140**, 35–51. (doi:10.1002/cne.901400103)
22. Voneida TJ, Mello NK. 1975 Interhemispheric projections of the optic tectum in pigeon. *Brain Behav. Evol.* **11**, 91–108. (doi:10.1159/000123627)
23. Manns M, Ströckens F. 2014 Functional and structural comparison of visual lateralization in birds: similar but still different. *Front. Psychol.* **5**, 206. (doi:10.3389/fpsyg.2014.00206)
24. von Fersen L, Güntürkün O. 1990 Visual memory lateralization in pigeons. *Neuropsychologia* **28**, 1–7. (doi:10.1016/0028-3932(90)90081-X)
25. Folta K, Diekamp B, Güntürkün O. 2004 Asymmetrical modes of visual bottom-up and top-down integration in the thalamic nucleus rotundus of pigeons. *J. Neurosci.* **24**, 9475–9485. (doi:10.1523/JNEUROSCI.3289-04.2004)
26. Andrew RJ, Mench J, Rainey C. 1982 Right-left asymmetry of response to visual stimuli in the domestic chick. *Analysis of Visual Behaviour* (eds DJ Ingle, MA Goodale, RJW Mansfield), pp. 225–236. Cambridge, MA: MIT Press.
27. Freeman R, Mann R, Guilford T, Biro D. 2011 Group decisions and individual differences: route fidelity predicts flight leadership in homing pigeons (*Columba livia*). *Biol. Lett.* **7**, 63–66. (doi:10.1098/rsbl.2010.0627)
28. Prior H, Wiltschko R, Stapput K, Güntürkün O, Wiltschko W. 2004 Visual lateralization and homing in pigeons. *Behav. Brain Res.* **154**, 301–310. (doi:10.1016/j.bbr.2004.02.018)
29. Gagliardo A, Odetti F, Ioalè P. 2005 Factors reducing the expected deflection in initial orientation in clock-shifted homing pigeons. *J. Exp. Biol.* **208**, 469–478. (doi:10.1242/jeb.01383)
30. Ulrich C, Prior H, Duka T, Leshchins'ka I, Valenti P, Güntürkün O, Lipp H-P. 1999 Left-hemispheric superiority for visuospatial orientation in homing pigeons. *Behav. Brain Res.* **104**, 169–178. (doi:10.1016/S0166-4328(99)00062-5)
31. Prior H, Lingenauber F, Nitschke J, Güntürkün O. 2002 Orientation and lateralized cue use in pigeons navigating a large indoor environment. *J. Exp. Biol.* **205**, 1795–1805.
32. Güntürkün O. 1985 Lateralization of visually controlled behavior in pigeons. *Physiol. Behav.* **34**, 575–577. (doi:10.1016/0031-9384 (85)90051-4)

Published Version of Chapter 4

The published typesetting of Chapter 4 begins on the following page. The citation for the chapter is as follows:

Martinho, Antone, Zackory T. Burns, Auguste MP von Bayern, and Alex Kacelnik. "Monocular Tool Control, Eye Dominance, and Laterality in New Caledonian Crows." *Current Biology* 24, no. 24 (2014): 2930-2934.

Current Biology 24, 2930–2934, December 15, 2014 ©2014 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2014.10.035

Report

Monocular Tool Control, Eye Dominance, and Laterality in New Caledonian Crows

Antone Martinho III,[1,3] Zackory T. Burns,[1,3] Auguste M.P. von Bayern,[1,2] and Alex Kacelnik[1,*]
[1]Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK
[2]Department of Behavioural Ecology and Evolutionary Genetics, Max Planck Institute for Ornithology, Eberhard-Gwinner-Straße, 82319 Seewiesen, Germany

Summary

Tool use, though rare, is taxonomically widespread, but morphological adaptations for tool use are virtually unknown [1]. We focus on the New Caledonian crow (NCC, *Corvus moneduloides*), which displays some of the most innovative tool-related behavior among nonhumans [2–6]. One of their major food sources is larvae extracted from burrows with sticks held diagonally [7] in the bill, oriented with individual, but not species-wide, laterality [8, 9]. Among possible behavioral [10] and anatomical adaptations for tool use [5, 11–15], NCCs possess unusually wide binocular visual fields (up to 60°), suggesting that extreme binocular vision may facilitate tool use [5]. Here, we establish that during natural extractions, tool tips can only be viewed by the contralateral eye. Thus, maintaining binocular view of tool tips is unlikely to have selected for wide binocular fields; the selective factor is more likely to have been to allow each eye to see far enough across the midsagittal line to view the tool's tip monocularly [5, 16]. Consequently, we tested the hypothesis that tool side preference follows eye preference and found that eye dominance does predict tool laterality across individuals. This contrasts with humans' species-wide motor laterality and uncorrelated motor-visual laterality [17], possibly because bill-held tools are viewed monocularly and move in concert with eyes, whereas hand-held tools are visible to both eyes and allow independent combinations of eye preference and handedness. This difference may affect other models of coordination between vision and mechanical control, not necessarily involving tools.

Results

View of the Tool's Tip

We modeled the geometry of natural food extractions, by combining morphological data [15], and the structure of visual fields [5] to compute the depths at which eyes see into foraging hollows as a function of hole diameter and probing distance (Figure 1; Supplemental Experimental Procedures available online). According to this model (Figure 1C), the eye contralateral to the tool's tip sees the tip independently of a hollow's diameter, but the ipsilateral eye's maximum depth of view depends on hole diameter and distance between bill's tip and hole's opening. In the notation of Figure 1C, binocular viewing requires that $B \geq \lambda$.

We compared the set of parameters for which this inequality holds true in natural circumstances by using ecologically relevant hole dimensions from Bluff et al. [18]. Sensitivity was examined by comparing the following three parameter ranges: one favoring binocularity, another favoring monocularity, and a third interpolating between these (see Experimental Procedures) [19–21].

For each parameter set, we plotted two volumes in a space of tool's tip depth, hole diameter, and distance between bill and burrow's opening (Figure 2). One volume shows the region for which binocular vision of the tool's tip is possible, and the other volume shows the ecologically relevant space. The intersection between the two volumes shows the ecologically relevant area where binocular vision of tool tips is possible. Figure 2 shows this graphically and quantitatively in separate tables. Even assuming an extreme ecological range most favorable to binocularity, a majority of extractions occur in circumstances compelling monocularity. Given our conservative modeling, this intersection is likely to be much smaller, meaning that food extraction overwhelmingly involves monocular viewing of the tool's tip.

Thus, the species's extremely wide visual fields may be an adaptation for tool use, but not through binocular control. Consistently, with a general argument put forward by Martin [16], for bird visual fields in general, the wide overlap between left and right visual fields in New Caledonian crows (NCCs) may be a consequence of allowing each eye to see far enough across the midsagittal plane to include the tip of obliquely held tools. The NCCs' strong individual laterality of tool use and lack of consistent species-wide bias may result from holding tools such that their tips fall in the field of the preferred eye. If this is true, eye preference should predict individual tool sidedness. We tested this prediction by examining whether the eye preferred by individual NCCs to explore potential hollows in the absence of available tools predicts individual tool-holding sidedness.

Eye Dominance and Laterality

Nine out of ten subjects completed an eye-dominance test. Out of these, four were right biased and five were left biased. These subjects also completed a tool laterality test, of which three were found to be right biased and six were found to be left biased. Eye preference reliably predicted tool sidedness (see Figure 3; n = 9, two-tailed binomial test, p = 0.039), with only one subject opposing the prediction (Figure 3 and Table S1).

Discussion

NCCs show strong individual lateralization of tool use, display no detectable population-level bias ([8, 9] and present data), have unusually wide overlap between the visual fields of both eyes, and prefer to hold their tools' working end on the side opposite to their preferred eye. By contrast, humans show species-level laterality of handedness and no correlation between the dominant eye and hand [17, 22], probably reflecting the difference between unity of movement between head and tools in crows, as compared to independence of movement between head and hands in humans.

[3]Co-first author
*Correspondence: alex.kacelnik@zoo.ox.ac.uk

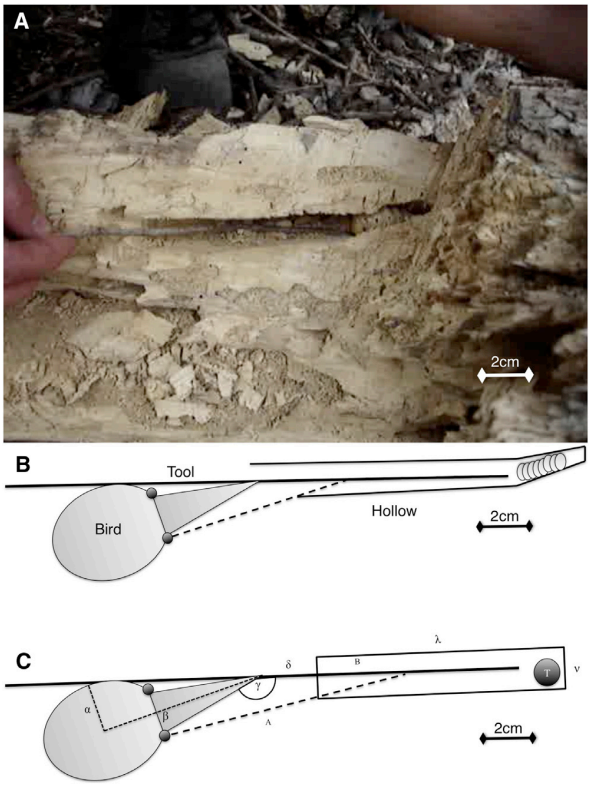


Figure 1. The Geometry of Vision and Tool Use in New Caledonian Crows

(A) Author (A.K.) probes a cross-sectioned larva burrow in a length of decaying candlenut wood.

(B) A simplified rendering of the larval burrow shown in Figure 1A, showing from above how a crow probes for larvae using a lateralized tool grip. The angled opening of the burrow results in a varying target depth of 7–10 cm.

(C) Top view of our model, generalized from Figure 1B, with a straight burrow and flat opening, showing a left-held tool being used to probe a cavity with a generalized target (T) at its bottom. The model identifies the obstructions of tool viewing by the eye on the side of the tool's tip. Using mean population morphology from Kenward et al. [15] for α and β, the angle of tool use, γ, can be calculated and used to determine the angle and depth of the tool tip's side eye's deepest line of sight into the hole, A. As δ (distance between bill's tip and burrow opening) decreases, B (maximum depth of tool visibility) also decreases, nonlinearly. Similarly, increases to the diameter of the hollow, ν, cause B to increase, while increases of target depth, λ, require a greater B for binocular viewing to be possible. As eye rotation and individual variation in the location of the eyes on the head cause varied interocular distance, we placed the eyes at the widest point of the bill, thus modeling the interocular distance as equivalent to bill width. This is the minimum possible interocular distance and biases the model in favor of binocular vision by increasing the depth at which the eye on the side of the tool's tip can see into the burrow.

Given that tool use is rarer than eye dominance—the latter affects multiple behavioral traits and taxa [23]—it is reasonable to infer that the causality goes from vision to tool sidedness rather than vice versa. The avian brain shows species-wide functional lateralization, and this may have been expected to cause consistent species-level laterality in eye dominance and tool use, but so far, neither consistency has been found. Alternatively, eye dominance could be a consequence of individuals compensating for differences in quality of vision between their eyes. Such remedial dominance is unlikely to have species-wide consistency but is consistent with eye dominance being causally related to tool use preference. Humans' eye dominance is about one-third left and two-thirds right, whereas handedness is approximately 90% right [17]. However, because humans and other primates manipulate tools with their hands, which can be moved independently of the head, differences between left and right eyesight do not need to constrain individuals' handedness. Crows instead are constrained to move tools and eyes in concert, and hence, a random distribution of left-right eye preference probably leads to individual, but not species-wide, side bias. Further, eyesight-determined laterality in tool use may act against the evolution of species-wide hemispheric specialization for tool control.

This line of thinking may be relevant to other forms of laterality, constrained or not by joint movement of body parts. For instance, at least one species studied so far, the Japanese jungle crow, *Corvus macrorhynchos*, appears to have species-level laterality of footedness across tasks [24]. The contrast with bill-controlled tool use may reflect that footedness in birds, like handedness in humans, is unconstrained by side differences in eyesight.

Experimental Procedures

Geometric Model

Modeling the geometry of tool-assisted extractions in ecological circumstances required estimates of six parameters. Three described anatomical traits (eye separation, bill and head length, and head width), and three were ecological variables that presented greater uncertainty (dimensions of probing holes, depth at which the tool tip operated, and distance between the NCCs' bill tip and the hole's opening). Some potentially relevant correlations are poorly known, in particular, the relationship between prey and hole dimensions. For instance, Rutz et al. [7] give the sizes of larva collected by humans in the NCCs' habitat as 3.6–7.6 cm in length and 1.0–1.6 cm in girth, but it is not known how representative these sizes are of the crows' diet or of the size of holes in which typical prey reside. We used information in the supplementary materials of Bluff et al. [18], describing the dimensions of holes in which tools were left behind by NCCs. With this information, we generated three sets of parameters, as indicated in Figure 2.

Operational Tool Tip Depth

NCCs extract beetle larva predominantly by provoking them to bite the tool tip, so both hole depth and position of the larva's head influence the operational location of the tool's tip. Bigger larvae reside in deeper burrows, but their greater length means that their heads are farther from the hole's bottom. As this correlation is unmapped, we used the hole depth quartiles provided by Bluff et al. [18]. They provide two sets of quartiles, one for twig tools and one for leaf stem tools. The lower pair of middle quartiles (4.3–9.3 cm) came from twig tools and favors binocularity, and the higher (5.0–12.4 cm), from leaf stem tools, favors monocularity. Averaging the extremes of both ranges gave us our interpolation range, 4.65–10.9 cm.

Hole Diameter

The hole openings described by Bluff et al. [18] had mean (±SD) minimum and maximum diameters of 1.392 cm ± 0.65 cm and 2.554 cm ± 1.25 cm, respectively. For the sensitivity analysis favoring binocularity, we used a range of 1 SD on either side of the mean of these diameters, estimating SD as the square root of the sum of the two variances, giving a range of 1.97 cm ± 1.40 cm. This range included both holes too narrow to contain a larva and holes of greater diameter than the average NCC's head [15], which would decrease the use of tools because the bird could easily access the prey with its bill [25]. We thus tightened our monocularity favoring range to span from the median minimum diameter, 1.3 cm, to the median maximum diameter, 2.4 cm, and held the median at 1.97 cm. The interpolation range was the midpoint between these two ranges.

Probing Distance

As bill-to-hole opening distance increases, the possibility of binocular view of tool tips increases. Tool length is not a suitable guide for this parameter because tools are most frequently held at intermediate points and inserted

A	Monocular biased
Hole diameter	1.3 – 2.4 cm
Target depth	5.0 – 12.4 cm
Probing distance	0.0 – 3.0 cm
Percent binocular	2.5%

B	Interpolation
Hole diameter	0.95 – 2.9 cm
Target depth	4.65 – 10.9 cm
Probing distance	0.0 – 5.25 cm
Percent binocular	24.9%

C	Binocular biased
Hole diameter	0.6 – 3.4 cm
Target depth	4.3 – 9.3 cm
Probing distance	0.0 – 7.5 cm
Percent binocular	45.3%

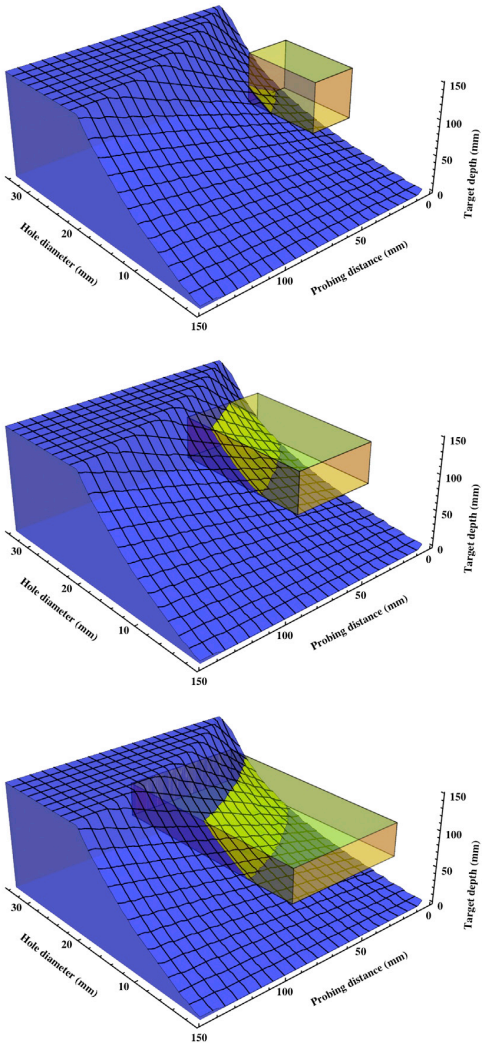


Figure 2. Ecological Relevance of Binocular and Monocular Vision

(A–C) Three sets of ecologically relevant ranges for hole diameter, target depth, and probing distance: one set of ranges favoring monocularity (A), one set favoring binocularity (C), and an interpolation obtained by taking the midpoint of the range limits from each of the other two sets (B). For an explanation of how each of these ranges was derived, see Experimental Procedures. Depicted are the foraging burrow dimensions for which binocular viewing of target are possible (blue volume, which is the same across plots) and ecologically relevant burrow dimensions and probing distances (yellow prism, based on the relevant set of ranges from the table above each plot). The dark area of intersection shows the combinations of cavity dimensions and bill distances for which binocular viewing is possible. The size of this intersection is represented in the table as the percent of cases within the ecologically relevant yellow prism that are also within the binocular blue volume. As the plots reveal, this intersection is relatively small, amounting to less than half of the ecologically relevant conditions, even under the parameters most favorable to binocularity. Thus, the majority of the crows' tool use occurs under monocular conditions.

at various depths, from zero at insertion time upward, resulting in bill tip-to-hole opening distances often shorter than 2 cm. We used 0–3 cm as the range favoring monocular viewing and 0–7.5 cm as the range favoring binocular viewing, including the insertion distance of above-average length tools. The selected interpolation range, 0–5.25 cm, was the midpoint of each value for these two ranges.

Subjects and Housing

Subjects were 13 wild caught NCCs (seven females and six males), with experimental histories. They were housed in groups of two or three in outdoor aviaries (60 m^2) with indoor enclosures (8 m^2), with ad libitum food for 12 hr per day and water at all times. Details of housing conditions can be found in von Bayern et al. [26]. All experiments complied with German animal experimentation legislation (§7 Bundestierschutzgesetz).

Tool Laterality Test

Tool laterality was determined following Weir et al. [8], except where otherwise noted. We used a semicircular tree stump section with two holes on its side, each 1.6 cm in diameter and 10 cm deep. A piece of doweling 0.3 cm in diameter and 20 cm long was furnished for use as a tool (Figure 4A). This doweling was longer than tools left behind in nature [18], as longer tools result in a higher incidence of lateralized tool use.

Testing occurred before the usual food supply was placed in the indoor aviary each morning, approximately 11 hr after ad libitum food had been removed the previous night. The probing holes were baited with chilled mealworms, with an additional mealworm near the openings to attract the birds' attention. The surface of the apparatus was sprinkled with sawdust to approximate the cues left by the NCCs' natural prey [27]. The log was placed in the enclosure approximately 10 cm from the center of a wall,

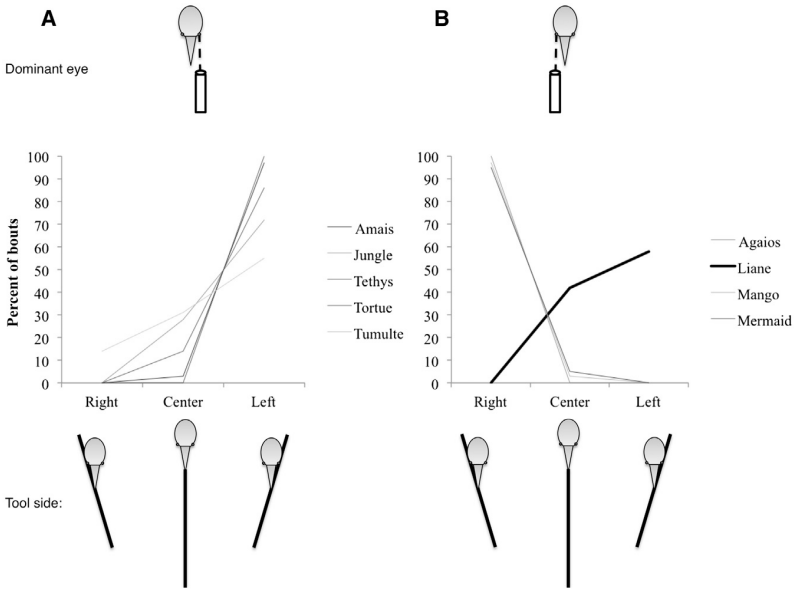


Figure 3. Percentages of Lateralized Tool Bouts for Left-Eye-Dominant and Right-Eye-Dominant Individuals

Percentages of lateralized tool bouts for left-eye-dominant (A) and right-eye-dominant (B) individuals. Each line represents an individual, showing the overall preference to hold the shaft of the tool on the same side as the dominant eye. Liane, the exception, is represented by the dark line in (B). Note her high percentage of center-held bouts.

with the doweling placed parallel to the wall, 10 cm in front of the apparatus. The test bird was then confined to the indoor enclosure and visually isolated from the outside aviary. Sessions were video recorded from above the apparatus and lasted 30 min from the time of presentation.

Laterality of tool use was scored in "bouts" of tool use [8]. A new bout started if the crow changed tool grip or released the tool and moved its head before regrasping it. Bouts were scored as "right" or "left," according to the cheek against which the nonfunctional end of the tool rested (Figure 4C), and "centered" if the tool was held straight. Tests were completed once the bird engaged in a minimum of ten lateralized bouts. If the subject did not achieve this in 30 min, it was retested once, and the bouts from both trials were pooled. To compute individual laterality, we excluded center-held bouts, as did Weir et al. [8]. The statistical significance of individual lateral bias was determined by a two-tailed binomial test against a random expectation of 50%.

Eye-Dominance Test

Subjects that completed the tool laterality test were tested for eye dominance in the same enclosure. The apparatus was a rectangular wooden board (15.5 cm × 21 cm), with an opaque plastic tube 8.5 cm tall and 1.6 cm internal diameter protruding from its center (Figure 4B). The tube contained a darkling beetle larva (*Zophobas morio*), and the board was sprinkled with sawdust and mealworms to attract the crow's attention [27]. The apparatus was placed 10 cm from a wall's midpoint. The subject was confined to the indoor enclosure after placement of the apparatus. Tests were video recorded from above.

In the absence of potential tools, the bait was out of the bird's reach. Seven subjects took one look down the tube, of which six then abandoned the apparatus and one attempted to upturn it. The remaining two looked more than once and attempted to retrieve the food. We operationally defined eye dominance by the first "look." This normalized analysis across

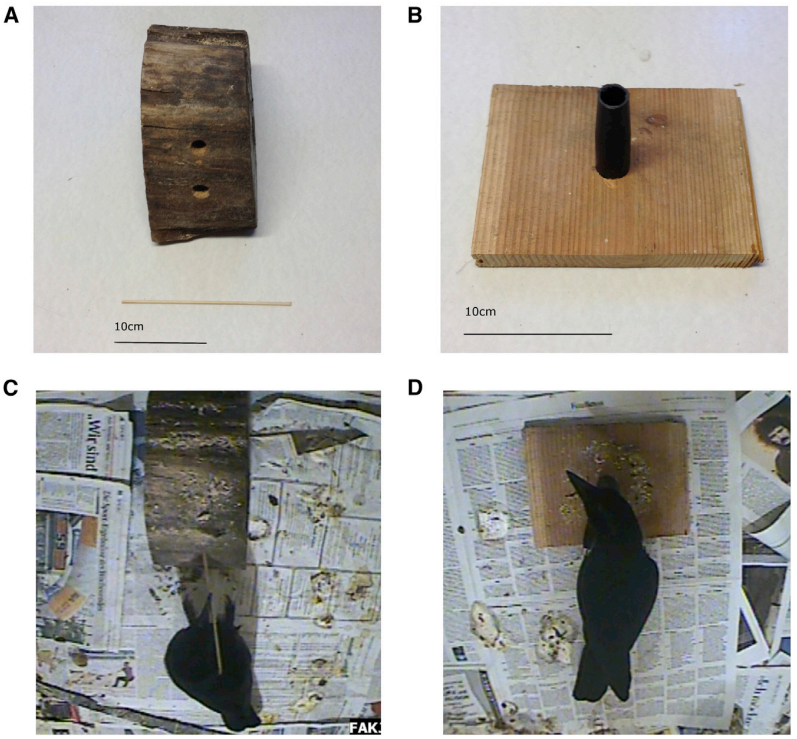


Figure 4. Experimental Apparatus

(A) Tool laterality probing apparatus and tool. Note the perpendicular placement of the tool to prevent approach bias.
(B) Eye-dominance apparatus.
(C) Right-sided tool use by Mango, viewed from above.
(D) A right-eye "look," with eye placed directly to the hole, by Liane, viewed from above.

subjects and controlled for the possibility that eye preference is expressed both in absolute use and in order of eye use. A look was defined by one eye being placed directly above and within a bill's length of the tube's opening (Figure 4D), ensuring a direct and uninterrupted line of sight to the tube's bottom.

Sessions lasted between 5 min and 30 min, ending 5 min after the first look. Although the testing room was searched prior to tests to eliminate potential tools, one crow (Agaios) retrieved a previously cached tool and approached the apparatus with it before having looked. We removed the tool once the bird dropped it and resumed testing. Each crow was tested until it looked once or failed to do so in four sessions. Four subjects were excluded from analysis because they did not look at the apparatus.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.10.035.

Author Contributions

A.M. and Z.T.B. conceived and designed the study in collaboration with A.M.P.v.B. and A.K. Data were collected by A.M. and Z.T.B. in collaboration with A.M.P.v.B., who was also responsible for the NCC colony. A.M. performed analyses, with support from Z.T.B. and A.K. A.M., Z.T.B., and A.K. wrote and edited the manuscript, with input from A.M.P.v.B.

Acknowledgments

We thank the authorities of Province du Sud, Nouvelle Calédonie and Christian Rutz for help in sourcing our subjects. A.M. and Z.T.B. were supported by the Oxford Department of Zoology Graduate Research Fund. We are grateful to Berenika Moduszewska, Lauriane Rat-Fischer, Jasmin Werner, and Irmgard Neumair for their expertise in the care and handling of the crows and for laboratory assistance. Kate Reynolds assisted with preparation of Figure 2. Our colleagues John Endler, Brian Rogers, Michael Haslam, Victoria Tobolsky, Jolyon Troscianko, and Dora Biro made constructive comments on previous versions of the manuscript.

Received: July 24, 2014
Revised: September 3, 2014
Accepted: October 13, 2014
Published: December 4, 2014

References

1. Shumaker, R.W., Walkup, K.R., and Beck, B.B. (2011). Animal Tool Behavior: The Use and Manufacture of Tools by Animals (Baltimore: JHU Press).
2. Hunt, G.R. (1996). Manufacture and use of hook-tools by New Caledonian crows. Nature *379*, 249–251.
3. Hunt, G.R., and Gray, R.D. (2003). Diversification and cumulative evolution in New Caledonian crow tool manufacture. Proc. Biol. Sci. *270*, 867–874.
4. McGrew, W.C. (2013). Is primate tool use special? Chimpanzee and New Caledonian crow compared. Philos. Trans. R. Soc. Lond. B Biol. Sci. *368*, 20120422.
5. Troscianko, J., von Bayern, A.M.P., Chappell, J., Rutz, C., and Martin, G.R. (2012). Extreme binocular vision and a straight bill facilitate tool use in New Caledonian crows. Nat Commun *3*, 1110.
6. Rutz, C., and St Clair, J.J.H. (2012). The evolutionary origins and ecological context of tool use in New Caledonian crows. Behav. Processes *89*, 153–165.
7. Rutz, C., Bluff, L.A., Reed, N., Troscianko, J., Newton, J., Inger, R., Kacelnik, A., and Bearhop, S. (2010). The ecological significance of tool use in New Caledonian crows. Science *329*, 1523–1526.
8. Weir, A.A.S., Kenward, B., Chappell, J., and Kacelnik, A. (2004). Lateralization of tool use in New Caledonian crows (Corvus moneduloides). Proc. Biol. Sci. *271* (*Suppl 5*), S344–S346.
9. Rutledge, R., and Hunt, G.R. (2004). Lateralized tool use in wild New Caledonian crows. Anim. Behav. *67*, 327–332.
10. Kenward, B., Weir, A.A.S., Rutz, C., and Kacelnik, A. (2005). Behavioural ecology: tool manufacture by naive juvenile crows. Nature *433*, 121–121.
11. Delacour, J. (1966). Guide des oiseaux de la Nouvelle-Calédonie et de ses dépendances (Neuchâtel: Delachaux et Niestlé SA).
12. Jollie, M. (1978). Phylogeny of the species of Corvus. Biologist *60*, 73–108.
13. Goodwin, D. (1986). Crows of the World (London: British Museum).
14. Doughty, C., Day, N., and Plant, A. (1999). Birds of the Solomons, Vanuatu & New Caledonia (London: A & C Black).
15. Kenward, B., Rutz, C., Weir, A.A.S., Chappell, J., and Kacelnik, A. (2004). Morphology and sexual dimorphism of the New Caledonian crow Corvus moneduloides, with notes on its behaviour and ecology. Ibis *146*, 652–660.
16. Martin, G.R. (2009). What is binocular vision for? A birds' eye view. J. Vis. *9*, 1–19.
17. Bourassa, D. (1996). Handedness and eye-dominance: a meta-analysis of their relationship. Laterality: asymmetries of body. Brain Cogn. *1*, 5–34.
18. Bluff, L.A., Troscianko, J., Weir, A.A.S., Kacelnik, A., and Rutz, C. (2010). Tool use by wild New Caledonian crows Corvus moneduloides at natural foraging sites. Proc. Biol. Sci. *277*, 1377–1385.
19. Wickham, H. (2011). The split-apply-combine strategy for data analysis. J. Stat. Softw. *40*, 1–29.
20. R Development Core Team (2013). R: A language and environment for statistical computing (Vienna: R Foundation for Statistical Computing).
21. Ligges, U., and Mächler, M. (2003). Scatterplot3d - an R package for visualizing multivariate data. J. Stat. Softw. *8*, 1–20.
22. Merrell, D.J. (1957). Dominance of eye and hand. Hum. Biol. *29*, 314–328.
23. Rogers, L.J. (2008). Development and function of lateralization in the avian brain. Brain Res. Bull. *76*, 235–244.
24. Izawa, E.-I., Kusayama, T., and Watanabe, S. (2005). Foot-use laterality in the Japanese jungle crow (Corvus macrorhynchos). Behav. Processes *69*, 357–362.
25. Taylor, A.H., Hunt, G.R., and Gray, R.D. (2012). Context-dependent tool use in New Caledonian crows. Biol. Lett. *8*, 205–207.
26. von Bayern, A.M.P., Heathcote, R.J.P., Rutz, C., and Kacelnik, A. (2009). The role of experience in problem solving and innovative tool use in crows. Curr. Biol. *19*, 1965–1968.
27. Troscianko, J. (2011). Ecological, morphological, and behavioural aspects of tool-use in New Caledonian crows. PhD thesis (Birmingham: University of Birmingham).

Published Version of Chapter 5

The published typesetting of Chapter 5 begins on the following page. The citation for the chapter is as follows:

Martinho, Antone, and Alex Kacelnik. "Ducklings imprint on the relational concept of "same or different"." *Science* 353, no. 6296 (2016): 286-288.

ACKNOWLEDGMENTS

This work was largely supported through an AXA (global investment and insurance group) Research Fund Fellowship. We are grateful to the National Centers for Environmental Information for providing access to the World Ocean Database and to all those who have contributed ocean data from the region surrounding the Antarctic Peninsula. The glacier-change data reported in this paper are tabulated in a database in the supplementary materials. We are most grateful to the reviewers who gave recommendations for improving this paper.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6296/283/suppl/DC1
Materials and Methods
Figs. S1 and S2
Table S1
Database S1
References (*33*)

4 December 2015; accepted 9 June 2016
10.1126/science.aae0017

EVOLUTIONARY COGNITION

Ducklings imprint on the relational concept of "same or different"

Antone Martinho III* and Alex Kacelnik*

The ability to identify and retain logical relations between stimuli and apply them to novel stimuli is known as relational concept learning. This has been demonstrated in a few animal species after extensive reinforcement training, and it reveals the brain's ability to deal with abstract properties. Here we describe relational concept learning in newborn ducklings without reinforced training. Newly hatched domesticated mallards that were briefly exposed to a pair of objects that were either the same or different in shape or color later preferred to follow pairs of new objects exhibiting the imprinted relation. Thus, even in a seemingly rigid and very rapid form of learning such as filial imprinting, the brain operates with abstract conceptual reasoning, a faculty often assumed to be reserved to highly intelligent organisms.

Relational concepts, such as "same" and "different," have been demonstrated in a few animal species, typically after extensive training (*1*, *2*). Relational concepts differ from other forms of categorical generalization. For instance, pigeons and bees can be trained to discriminate whether novel images contain humans or not (*3*), or whether novel paintings are by Monet or Picasso (*4*), by relying on the similarity between features of the training and of the novel stimuli. In relational concept learning, however, relative properties between training stimuli generate the relationship that has to be generalized to sets of novel stimuli (*5*).

The relations of "same" and "different" have been used to study relational concept learning in a few primates and birds (*6*), using a variety of protocols. For instance, in the identity matching to sample (IMTS) protocol, an animal sees a sample stimulus and subsequently chooses between two test stimuli, one of which is identical to the sample. Reinforcement can be contingent on responding to the identical one ("same") or to the alternative ("different"). Honey bees can learn this discrimination and even transfer a correct response to novel stimuli across sensory modalities (olfaction and visual texture) (*7*). The IMTS task requires learning the appropriate comparison between the working-memory representation of the sample and the currently perceived test stimuli, but it does not require interpreting an abstract relationship between perceived items and then reapplying the same relation to discriminate between sets of novel objects.

A different procedure, that isolates relational learning, involves presenting more than one stimulus as a sample, and then selecting, from between various sets of stimuli, the set that has

Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.
***Corresponding author. Email: antone.martinho@zoo.ox.ac.uk (A.M.); alex.kacelnik@zoo.ox.ac.uk (A.K.)**

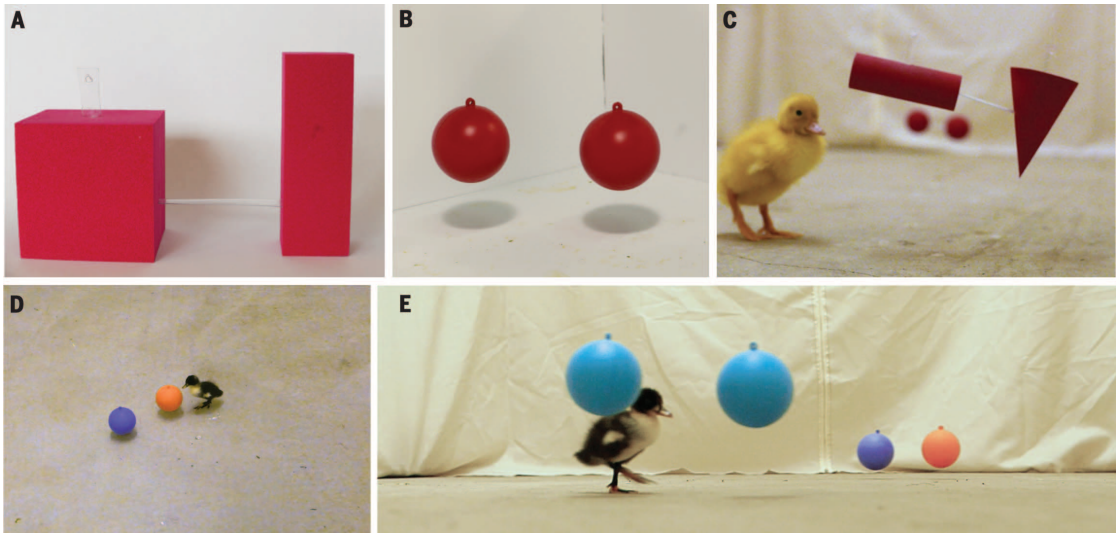


Fig. 1. Imprinting and testing stimuli. Newborn ducklings were first exposed to a pair of objects revolving about the center of a training arena, then tested with two novel pairs of objects. (**A**) Example of a "different shape" training stimulus pair. (**B**) Example of a "same color" training stimulus pair. After this exposure, the ducklings were tested for their preference between two novel stimulus pairs revolving in apposition. (**C**) A duckling trained with the set shown in (A) demonstrates its preference for a novel "different shape"' stimulus over an also-novel "same shape" stimulus. (**D**) A duckling trained with the stimulus pair shown in (B) approaches a novel "different color" stimulus pair—an incorrect response. (**E**) The same duckling later in the same trial correctly approaches and closely follows the novel "same color" stimulus.

the same internal relation as the sample set, a procedure called relational matching to sample (RMTS) (*1*, *8*). In RMTS, what has to be retained is a relation between stimuli, rather than a representation of a perceived stimulus. Primates (*9*), pigeons (*5*), parrots (*10*), and corvids (*1*) have succeeded in solving such problems, and it has been cogently argued that this may indicate their possession of the ability to reason by analogy (*8*).

Demonstrating the capacity for both identity and relational matching to sample has so far used reinforcement, and often extensive training, to allow subjects to infer the target relation. This contrasts with avian filial imprinting, a specialized form of unrewarded learning by which hatchlings acquire the ability to identify and then follow a parent or substitute parental object (*11*–*13*). As could be expected from its biological adaptive significance, imprinting is one of the fastest and most reliable forms of learning (*14*). In chicks and ducklings, high-fidelity imprinting responses can be acquired in a few minutes of unrewarded exposure to a stimulus (*15*).

Filial and sibling imprinting cannot be mediated just by snapshot representations of two-dimensional retinal images. Wood summarized the problem sharply: "Building an invariant object representation requires transforming patterns of retinal activity (view-specific information) into an abstract representation that is tolerant to retinal image changes and selective for a particular object (identity information)" (*16*). This is particularly relevant when considering that ducklings may benefit by recognizing not just their mother but also their group of siblings (*17*), because broods may have different degrees of heterogeneity. Evidence showing that young birds are sensitive to abstract qualities of stimuli, both spontaneously and through imprinting, supports this view and shows that imprinting is far richer, as a learning phenomenon, than had originally been envisaged (*18*–*21*). It is thus tempting to ask whether their abstraction abilities may extend to the more demanding phenomenon of relational concept representation, which has so far only been demonstrated through extensive reinforcement in species with advanced intelligence. To this effect, we modified the RMTS protocol to combine it with imprinting, as follows.

Following Bateson's (*22*) and Lickliter's (*23*) procedures for effective imprinting, we hatched domesticated mallard ducklings in the dark and kept them for 1 hour in a social group, with light, food, and water. They were then exposed for 25 min to a moving pair of sample objects, kept for a 30-min retention interval in the dark, and presented with two novel pairs of moving objects for 10 min (Fig. 1). Sample stimuli within the pair shown in the imprinting phase were either equal to each other in both color and shape, or different in one of these characteristics. Test stimuli in the preference test consisted of two stimuli pairs, composed of objects novel to the birds. In one test pair, the objects were equal in color and shape, and in the other they differed in either shape or color (Fig. 2). Detailed methods may be found in the supplementary materials.

In experiment 1, the imprinting phase stimulus pair consisted of two red solids, which were equal to each other in shape for group "same" and different for group "different" (n = 36 ducklings for each group). The stimuli forming the two test pairs were also red, but one pair consisted of two novel, identical shapes and the other of two novel, different shapes. In experiment 2, the imprinting stimulus pair was two spheres, either of the same color or different colors (n = 40 ducklings for each group), and test stimuli were also spherical but had novel colors, equal in one pair and different in the other.

To measure preference, the number of approaches undertaken by each duckling toward each test pair was scored twice, once by an

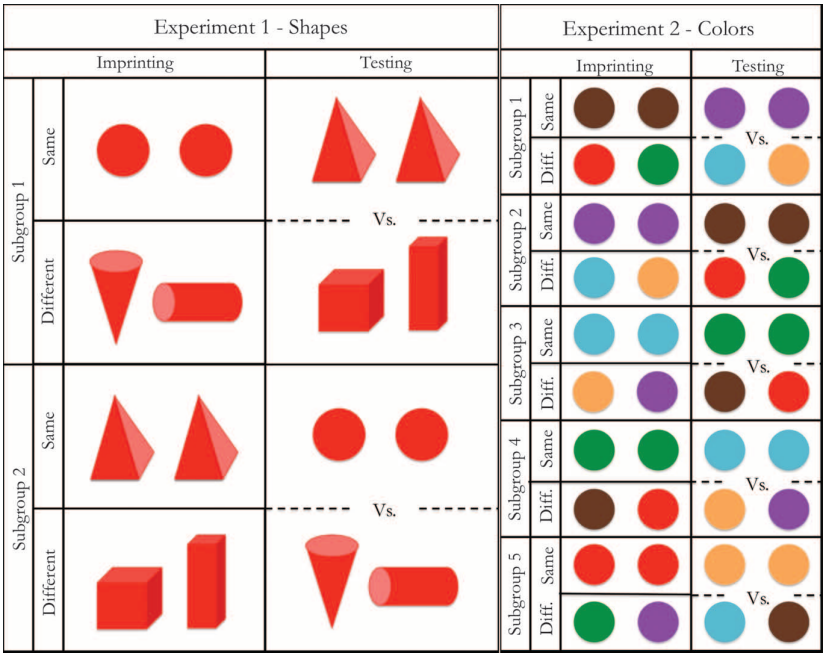


Fig. 2. Experimental stimulus pairs. Experiment 1 tested for responses between red objects that could differ in shape, and experiment 2 tested for responses between spherical objects that could differ in color, using the pairs illustrated here. In experiment 1, members of subgroup 1 were initially exposed to either two spheres or a pair formed by a cone and a cylinder and were then tested for preference between a pair of two pyramids and a pair formed by a prism and a cube, whereas members of subgroup 2 were imprinted on pairs of either two pyramids or a prism and a cube and were then tested on preference between a pair of spheres and a pair formed by a cone and a cylinder. In experiment 2, members of each subgroup were trained on either two identical spheres or a pair formed by two spheres of different colors, and all ducklings were tested for preference between two novel identical spheres and a pair formed by two novel differing spheres.

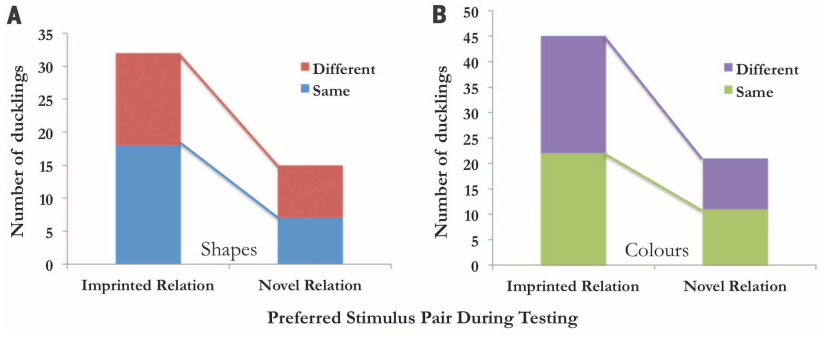


Fig. 3. Ducklings' preferences for an imprinted relational concept. The number of ducklings showing preference for the imprinted (left column) or alternative (right column) relation is shown for shape relations in experiment 1 (**A**) and the same for color relations in experiment 2 (**B**). Ducklings preferred the imprinted relation in both shape and color, regardless of whether the imprinted relation was "same" or "different."

independent scorer blind to each duckling's imprinting condition and to the study's hypothesis. Ducklings that were inactive during testing (fewer than five approaches) were excluded from analysis. Preferences were assessed via sign test, with sample size being the number of individual ducklings. Ducklings making more than half of their approaches toward a given stimulus were scored as having preferred it (see the methods in the supplementary materials). Video of sample trials (movie S1) is available in the supplementary materials.

Figure 3 shows the preference results. In experiment 1, out of a total of 47 active ducklings, 32 preferred the pair bearing their imprinted shape relation (two-tailed binomial test, $P = 0.02$). In experiment 2, out of 66 active ducklings, 45 preferred the stimulus pairs bearing their imprinted color relation (two-tailed binomial test, $P = 0.004$). Combining both results, out of 113 active ducklings, 77 preferred the relational concept, same or different, upon which they had imprinted (two-tailed binomial test, $P < 0.0001$).

The accuracy of our ducklings was comparable to, or better than, reinforced relational concept discrimination in primates (*24*) and crows (*1*). This finding supports a richer emerging view of the representation of information in the animal brain than is presently prevalent, in which even relatively simple learning systems do not process information just through the content of sensory signals but also by encoding higher-level, abstract aspects of stimulus analyses, already the target of neural network models designed to simulate such cognitive function (*25*). The ducklings' performance indicates that their brains may be prepared, not just to respond differentially to certain visual inputs, such as scrambled objects containing species-specific elements like legs or heads or virtual points that move in a biologically plausible coordination (*20*), but also to pick up abstract relational properties between elements of their sensory input and those elements' characteristics.

For young precocious birds, having this competence makes biological sense. For a duckling critically dependent on proximity to its mother and siblings, defining the attachment stimulus configuration as a library of sensory inputs and logical rules increases the likelihood that the mother and sibling group will be identified with high fidelity in spite of considerable variations in how they are perceived. The rules that may define the imprinted attachment target are likely to extend beyond properties of a single object such as color, shape, or symmetry, to include properties of object assemblies such as their informational entropy (*26*).

REFERENCES AND NOTES

1. A. Smirnova, Z. Zorina, T. Obozova, E. Wasserman, *Curr. Biol.* **25**, 256–260 (2015).
2. E. A. Wasserman, *Psychol. Bull.* **113**, 211–228 (1993).
3. R. J. Herrnstein, D. H. Loveland, C. Cable, *J. Exp. Psychol. Anim. Behav. Process.* **2**, 285–302 (1976).
4. W. Wu, A. M. Moreno, J. M. Tangen, J. Reinhard, *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **199**, 45–55 (2013).
5. T. R. Zentall, M. Galizio, T. S. Critchfied, *J. Exp. Anal. Behav.* **78**, 237–248 (2002).
6. A. A. Wright, J. S. Katz, *Behav. Processes* **72**, 234–254 (2006).
7. M. Giurfa, S. Zhang, A. Jenett, R. Menzel, M. V. Srinivasan, *Nature* **410**, 930–933 (2001).
8. R. G. Cook, E. A. Wasserman, *Psychon. Bull. Rev.* **14**, 1107–1114 (2007).
9. E. A. Wasserman, J. Fagot, M. E. Young, *J. Comp. Psychol.* **115**, 42–52 (2001).
10. I. M. Pepperberg, *Anim. Learn. Behav.* **15**, 423–432 (1987).
11. K. Lorenz, *J. Ornithol.* **83**, 289–413 (1935).
12. P. Bateson, *Anim. Behav.* **27**, 470–486 (1979).
13. P. Bateson, *Anim. Learn. Behav.* **7**, 259–262 (1979).
14. P. P. G. Bateson, *Biol. Rev. Camb. Philos. Soc.* **41**, 177–211 (1966).
15. P. Bateson, J. B. Jaeckel, *Anim. Behav.* **24**, 386–390 (1976).
16. J. N. Wood, *Dev. Sci.* **18**, 194–205 (2015).
17. E. H. Hess, D. B. Hess, *Psychon. Sci.* **14**, 129–130 (1969).
18. O. Rosa-Salva, L. Regolin, G. Vallortigara, *Dev. Sci.* **13**, 565–577 (2010).
19. G. Vallortigara, L. Regolin, F. Marconato, *PLOS Biol.* **3**, e208 (2005).
20. L. Regolin, L. Tommasi, G. Vallortigara, *Anim. Cogn.* **3**, 53–60 (2000).
21. R. Rugani, L. Regolin, G. Vallortigara, *Dev. Sci.* **13**, 790–797 (2010).
22. P. P. G. Bateson, A. A. P. Wainwright, *Behaviour* **42**, 279–290 (1972).
23. R. Lickliter, G. Gottlieb, *J. Comp. Psychol.* **101**, 40–46 (1987).
24. J. S. Katz, A. A. Wright, J. Bachevalier, *J. Exp. Psychol. Anim. Behav. Process.* **28**, 358–368 (2002).
25. P. Bateson, G. Horn, *Anim. Behav.* **48**, 695–715 (1994).
26. T. R. Zentall, E. A. Wasserman, O. F. Lazareva, R. K. Thompson, M. J. Rattermann, *Comp. Cogn. Behav. Rev.* **3**, 13–45 (2008).

ACKNOWLEDGMENTS

We thank the Oxford University Farm for allowing us to use their ducklings for this work, and Poultry Manager M. Colley for providing duckling husbandry and technical help. P. Bateson, E. Wasserman, and H. Smithson gave us valuable advice and recommendations regarding our methods and interpretation. We are also grateful to E. Truswell, who assisted with some experimental trials, and M. Lewis, who acted as an independent "blind" scorer for data analysis. This research was funded with assistance from the University of Oxford Department of Zoology. Materials and methods and a video of sample trials may be found in the supplementary materials. Video recordings of training and testing can be made available on request. Data obtained from video coding are available as supplementary materials on *Science* Online (table S1). The experiment was devised jointly by A.M. and A.K. Experimentation, data collection, and analysis were performed by A.M. with the advice and support of A.K.. The manuscript was written jointly by A.M. and A.K., and figures were prepared by A.M. and edited by A.K.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6296/286/suppl/DC1
Materials and Methods
Table S1
Movie S1
Reference (*27*)

5 February 2016; accepted 17 May 2016
10.1126/science.aaf4247

BIODIVERSITY

Has land use pushed terrestrial biodiversity beyond the planetary boundary? A global assessment

Tim Newbold,[1,2]* Lawrence N. Hudson,[3] Andrew P. Arnell,[1] Sara Contu,[3] Adriana De Palma,[3,4] Simon Ferrier,[5] Samantha L. L. Hill,[1,3] Andrew J. Hoskins,[5] Igor Lysenko,[4] Helen R. P. Phillips,[3,4] Victoria J. Burton,[3] Charlotte W. T. Chng,[3] Susan Emerson,[3] Di Gao,[3] Gwilym Pask-Hale,[3] Jon Hutton,[1,6] Martin Jung,[7,8] Katia Sanchez-Ortiz,[3] Benno I. Simmons,[3,4] Sarah Whitmee,[2] Hanbin Zhang,[3] Jörn P. W. Scharlemann,[1,8] Andy Purvis[3,4]

Land use and related pressures have reduced local terrestrial biodiversity, but it is unclear how the magnitude of change relates to the recently proposed planetary boundary ("safe limit"). We estimate that land use and related pressures have already reduced local biodiversity intactness—the average proportion of natural biodiversity remaining in local ecosystems—beyond its recently proposed planetary boundary across 58.1% of the world's land surface, where 71.4% of the human population live. Biodiversity intactness within most biomes (especially grassland biomes), most biodiversity hotspots, and even some wilderness areas is inferred to be beyond the boundary. Such widespread transgression of safe limits suggests that biodiversity loss, if unchecked, will undermine efforts toward long-term sustainable development.

Land use and related pressures have been the main drivers of terrestrial biodiversity change (*1*) and are increasing (*2*). Biodiversity has already experienced widespread large net losses (*3*), potentially compromising its contribution to resilient provision of ecosystem functions and services, such as biomass production and pollination, that underpin human well-being (*4*–*7*). Species-removal experiments suggest that loss of ecosystem function accelerates with ongoing species loss (*5*), implying that there may be thresholds beyond which human intervention is needed to ensure adequate local ecosystem function (*8*, *9*). The loss of 20% of species—which affects ecosystem productivity as strongly as other direct drivers (*5*)—is one possible threshold, but it is unclear by which