only in chlorophytes. Multilayered structures are found in chlorophytes and streptophytes, and appear to be an ancestral character of algae, as similar structures have been described in other algal groups[6].

Methods

DNA sequencing

Chloroplast DNA from *Mesostigma viride* (NIES-296) was isolated from total cellular DNA as an AT-rich fraction by CsCl-bisbenzimide isopycnic centrifugation[20]. This DNA preparation was sheared by nebulization, and 1,500–3,000-bp fragments were recovered by electroelution after agarose gel electrophoresis. These fragments were treated with *Escherichia coli* Klenow fragment and T7 DNA polymerase, and cloned into the *Sma*I site of Bluescript II KS+. After hybridization of the clones with the original DNA used for cloning, DNA templates from positive clones were prepared with the QIAprep 8 Miniprep kit (Quiagen). Nucleotide sequences were determined with the PRISM dye terminator cycle sequencing kit (Applied Biosystems) on a DNA sequencer (model 373; Applied Biosystems) using T3 and T7 primers. Sequences were assembled and analysed as described[20]. Short genomic regions not represented in the clones analysed were sequenced from PCR-amplified fragments.

Phylogenetic analysis

Genome sequences were retrieved from GenBank. *Pedinomonas* cpDNA sequences are from our unpublished data. Individual protein and rRNA gene sequences were aligned with CLUSTALW 1.74 (ref. 21), alignments were concatenated, and ambiguously aligned regions containing gaps were excluded. The alignments and data sets are available in Supplementary Information. The program packages MOLPHY 2.3b3 (ref. 16), PHYLIP 3.573c[22], PUZZLE 4.0.2[23] and SPLITSTREE 2.4[24] were used for phylogenetic analyses. Symmetric distance matrices were computed with PUZZLE and PROTDIST[22], whereas Logdet distances were calculated with SPLITSTREE. Distance trees were constructed with NEIGHBOR[22] and/or FITCH[22], maximum-parsimony trees were obtained with PROTPARS[22], and quartet-puzzling trees were generated with PUZZLE. The robustness of distance and maximum-parsimony trees was assessed by bootstrap percentages after 100 replications. In the case of quartet-puzzling trees, reliability percentages of the occurrence of the nodes were estimated after 10,000 puzzling steps. Maximum-likelihood analyses of protein and DNA sequences were carried out with PROTML[16] and NUCML[16], respectively, and local bootstrap probability was estimated by resampling of the estimated log likelihood[16].

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Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

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Pattern recognition and active vision in chickens

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Recognition of objects or environmental landmarks is problematic because appearance can vary widely depending on illumination, viewing distance, angle of view and so on[1]. Storing a separate image or 'template' for every possible view requires vast numbers to be stored and scanned, has a high probability of recognition error and appears not to be the solution adopted by primates[2,3]. However, some invertebrate template matching systems can achieve recognition by 'active vision' in which the animal's own behaviour is used to achieve a fit between template and object[4], for example by repeatedly following a set path[5–7]. Recognition is thus limited to views from the set path but achieved with a minimal number of templates. Here we report the first evidence of similar active vision in a bird, in the form of locomotion and individually distinct head movements that give the eyes a similar series of views on different occasions. The hens' ability to recognize objects is also found to decrease when their normal paths are altered.

When leaving a nest or newly discovered food source, bees and wasps use special orientation flights and then attempt to recapitulate the same set of views on subsequent occasions by following similar flight paths[5–7]. The functional significance of such active vision[4] is that by learning only a limited set of views of an object, the task of recognizing it is greatly simplified, although at the cost of recognition failure if the object is viewed from an unfamiliar

viewpoint. So far, no clear evidence for the importance of active vision in visual recognition has been documented for vertebrates[8]. Here we report the first evidence, to our knowledge, that object recognition in a bird also involves recapitulating similar views of objects on different occasions by repeated patterns of locomotion and head movements, and a failure of recognition from other viewpoints. We used frame-by-frame video analysis of the body and head movements of six freely moving hens discriminating between two stimulus objects for a food reward under three sets of conditions: (1) when the absolute position of the objects was varied from trial to trial but the birds nevertheless altered their behaviour to give themselves similar viewpoints; (2) when the appearance of the objects was altered slightly and the birds spent longer fixating them from their preferred viewing points; and (3) when a barrier prevented the hens from taking their usual path to the objects.

As stimuli, we used white blocks of wood with three-dimensional shiny ornaments (wing nuts, paper clips, bolts or nuts) glued onto the surface. The blocks were placed in front of two low walls, one of which hid a dish of food. The six hens had to learn which surface ornaments indicated the wall with food behind it, the position of the food (left or right) being varied from trial to trial on a pseudo-random (Gellerman) schedule. For condition (1), eight trials from each of six hens were filmed with an overhead video camera running at 25 frames per second. Videotapes were subsequently analysed by taking x and y coordinates for mid-head, beak and (front middle) object position for each frame, and then calculating the distance and head angle to both positive and negative stimulus objects. For condition (2), we interspersed a further set of 12 normal trials with 8 probe trials in which these familiar objects were substituted by similar ones in which the surface ornaments on either the front face or the side faces had been removed. For condition (3), we inserted a long barrier (120 cm) between the two stimulus objects so that the birds could not follow their usual path; in eight trials they were forced to make a choice at a much larger distance (120 cm) than normal.

All six hens developed fixed patterns of going round the objects to get at the food behind the relevant wall, regardless of whether the positive object was to the left or the right of the room. In the last 30 trials with training objects (mean was 85% correct), 3 hens always moved around the right-hand side of the stimulus object as they approached it, keeping it on their left in 100% of the trials; 2 hens moved around to the left side, leaving it on their right in 100% of the trials; and 1 hen kept the positive object on its left in over 90% of the trials (number of hens always keeping stimulus object on the same side on at least 90% of trials was 6/6; $P = 0.016$, binomial test). Even more strikingly, each hen showed her own individually distinct pattern of head movements that were very similar from trial to trial but often distinct between hens (Fig. 1a, b). Two birds used predominantly the left eye, two used the right eye, and two switched from one to the other in a predictable sequence. A 'fixation' was defined as a sequence of two or more frames (>80 ms) in which both eye position and head angle were constant[9,10]. All six hens fixated at individually distinct distances from the stimulus (Fig. 2), with a particular tendency to fixate at around 19–22 cm, a distance previously found to have significance in social interactions[11]. However, they showed no such consistency for the absolute position in space at which the fixations occurred (number of hens showing fixations at the same distance in at least 5/8 trials was 6/6, $P = 0.016$; number of hens showing fixations at the same head angle in 5 or more trials was 6/6, $P = 0.016$; and number of hens showing fixations at the same x coordinate in 5 or more trials was 1/6 and fixations at same y coordinate was 0/6). Their view of a stimulus was thus consistently from the same distance, with the same eye and the same head angle, but they did not fixate the stimuli from particular angles. When stimulus objects had ornaments removed from one or more of their faces, the hens showed an increase in the duration of

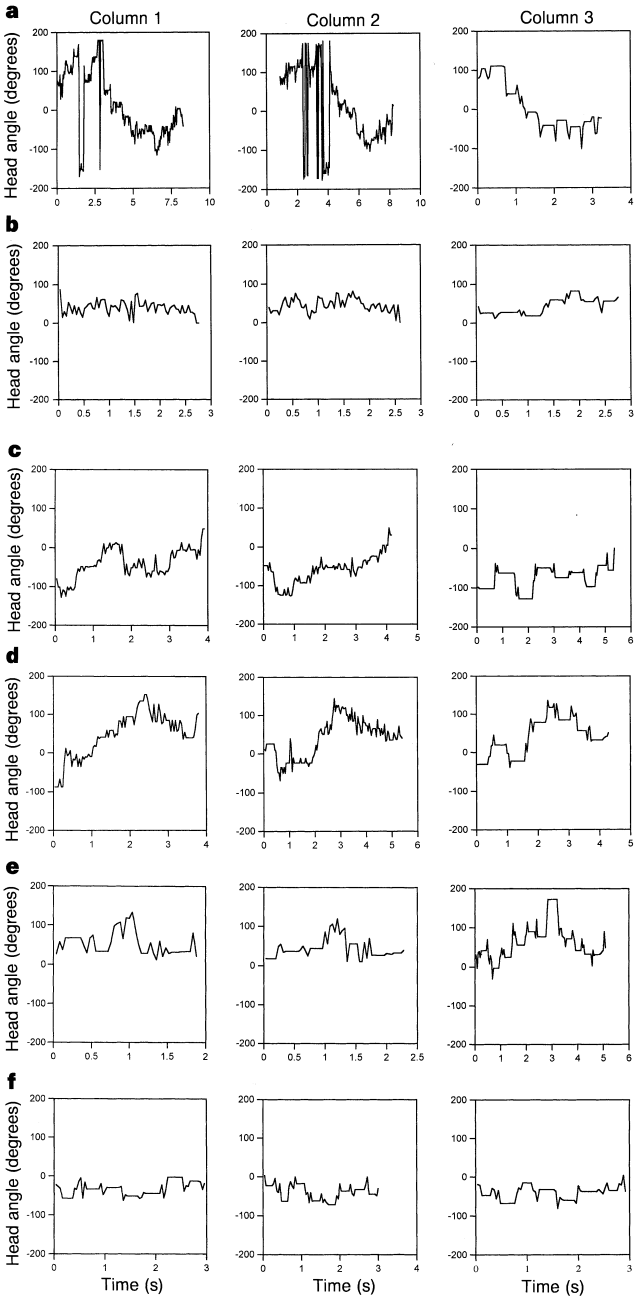

Figure 1 Examples from each of six hens of the head movements on approach to stimulus objects. Movements were recorded every 1/25 second from videotapes. Positive head-angle values indicate that a bird's left eye was pointing towards the positive object; negative angles indicate the right eye. Rows **a–f** indicate hens 1–6, respectively. Columns 1 and 2 show two examples of behaviour from each bird during trials with 'normal' stimuli seen throughout their training. Time 0 is the time at which the bird's head appeared in the video frame approximately 60 cm from the stimuli. Note the striking similarity of the two records for each bird. Only two records per bird are shown for illustration but statistical analyses were done using eight records for each bird. Each sequence was described by six parameters: intercept, linear, quadratic and cubic regression coefficients and a one-lag autocorrelation coefficient to account for serial dependence in the data. We used the transformed head angle (log (angle + 180)), as the response variable and obtained the estimates of the six coefficients and their standard errors using a linear mixed-effects model. For the mean head angle (indicating which eye was used), there were significant individual differences between hen 3 and the rest, and between hens 2 and 6 ($P < 0.001$). Column 3 shows , for each individual hen, an example of the head movements when the surface ornaments on the front face of the block were removed. There was an increase in duration of 'fixations' (sequences of two or more video frames in which the eye and head angle remained the same). These appear as plateaux on the graph.

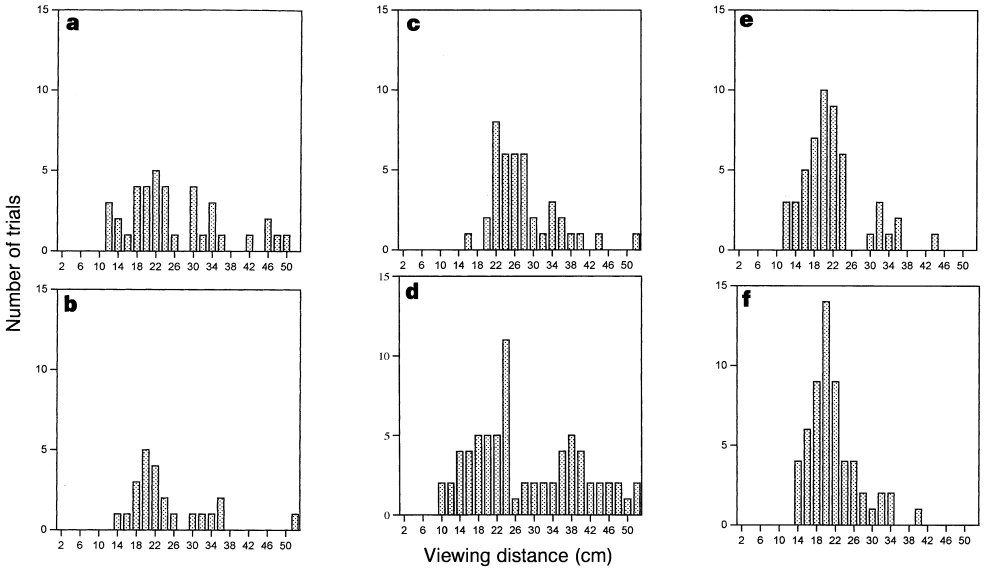

Figure 2 Frequency distribution of fixations at different distances. Shown are fixations (sequences in which the hen's eye position and head angle remained the same for at least 80 ms) that were repeated in at least 5 of 8 trials for the 6 hens. The graphs show that each hen had individually distinct preferred viewing distances, but that all individuals tended to include 19–22 cm as one of these distances.

fixations (shown by the plateaux in Fig. 1c) from a median of 110 ms in training trials to 310 ms ($P < 0.01$, $n = 6$, $P < 0.01$, Wilcoxon Matched Pairs Test). However, the hens did not consistently use one eye or the other to fixate the changed objects. Rather, they fixated with the eye that they were using at that point in their sequence of head movements (Fig. 1c). Hens using solely the left eye (hens 2 and 5) fixated with the left eye, hens using mainly the right eye (hens 3 and 6) fixated with the right eye, and hens that switched from right to left (hen 4) or left to right (hen 1) fixated with both in sequence. With the long barrier that prevented the birds from taking their normal path, their ability to discriminate fell significantly. All six hens showed lower discrimination with the long barrier (median was 50%) than with the short barrier (median was 90%; $P < 0.01$, $n = 6$, Wilcoxon Matched Pairs Test). All six hens showed significant discrimination of over 70% with the short barrier; only one showed discrimination of over 70% with the long barrier).

These results show that the eyes of an individual hen followed paths through time: an individual hen always went around the same side of an object, exhibited a similar pattern of head movements as it approached an object, and fixated at particular distances, viewing with the same eye and the same head angle on different trials. The visual significance of these individually distinct paths was confirmed by the finding that if the object had an unexpected appearance, the birds increased the amount of time fixating and then moved closer to look again, using the same eye and the same fixation distances as they had previously. Although both eye and head movements stabilize gaze in birds[9,10], eye movements are small (maximum 15°)[12]; similarity of head angle thus indicates similarity of eye angle within these limits[13]. Although the hens may have been looking with the same part of the eye, the eyes themselves did not follow the same path through space on different trials, so the hens did not fixate from particular viewpoints, as reported for bees and wasps[5–7] . However, the stimuli used here were not complex landmarks but decorated blocks that could be recognized by simple key features rather than more complex, angle-dependent 'snapshots'[5]. The results are consistent with the idea that birds use active vision for object recognition, in the form of precisely repeated locomotory and head movements that allow them to recapitulate views of objects from the same distance and with the same part of the eye on successive occasions. □

Methods

Six ISA Brown hens aged between 30 and 50 weeks were individually trained and tested in a room 1.8 × 3.0 × 2.0 m high. At one end of the room was a white wooden starting box (400 × 430 × 480 mm high), which had both a wooden and a clear perspex door. At the other end of the room, 1.8 m away, were two concrete walls (200 × 100 × 400 mm high) and 800 mm apart. A barrier (710 cm high) down the mid-line of the room extended 5 cm in front of the stimulus objects and separated the two walls. Which wall hid food was varied from trial to trial, but was always indicated by the presence in front of the wall of a white rectangular block of wood (75 × 75 × 200 cm high) which had 3 small shiny ornaments glued onto each face. (These were either 3 × 2-cm wing nuts one above the other on the front face and 3 × 2.75-cm bolts on each of the side faces or 3 × 2.5-cm safety pins on the front face and 3 × 1-cm bolts on each side face). Food (a mixture of grain and commercial layers mash) was placed in a small dish behind one of the walls. Each wall had cardboard baffles behind it so that an approaching hen could not see whether any food was present until she had gone around the back of it. The front of each brick was covered with bright red card to attract the attention of the birds, and in front of this card, the white wooden blocks were placed on stands so as to be level with the birds' eyes as they approached.

Before a training session, a hen would be taken out of the home pen and confined for one hour in a small cage with water but no food and within sight of its home group of seven other hens. This short period of food deprivation was necessary to motivate the birds to run the trials, and the confinement in a separate cage was so that food did not have to be taken away from all the birds in the home group.

The six hens were first trained to come out of the starting box and go behind a wall (varied between being the one on the left and the one on the right in a Gellerman sequence to avoid the birds developing position preferences) to find food. They were given 8–10 trials per day. For each trial, a hen was confined in the starting box with both doors down. The wooden door was then raised, giving the hen a view of the walls and their associated objects. After 20 seconds, the perspex door was also raised and the hen was free to walk out. If she went behind the wall with the positive stimulus in front of it, she was allowed to feed for 10 seconds and was then picked up by hand and returned to the starting box. For each trial, the hen's choice and which direction she went round the wall to look for food was recorded.

For pre-training, the relevant wall had the positive stimulus in front of it and the other wall had nothing. Once the hens had learned this task, they were presented with both the positive and the negative stimulus and training was continued until the hens had achieved a criterion of 3 successive days with 8 or more of the 10 daily trials correct. This occurred for all hens within between 5 and 10 days. Training was then continued for approximately 10 more days before video recordings were made.

An overhead CCTV camera was positioned 2 m above the floor in the middle of the room and behaviour recorded using a Panasonic VHS recorder. The resulting video tapes were played into an AV Power Mac and analysed frame-by frame (25 frames per second). For each frame, the x and y coordinates of the position of the middle of the bird's head (between the eyes), its beak tip and the position of the front centre of the positive and negative stimulus objects were recorded with a mouse pointer. These coordinates were then used to calculate the distance from the birds' eye to the stimulus objects and the head angle (the angle between a line drawn from the middle of the bird's head to its beak tip and a line drawn from the head to the object.

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Altered brain response to verbal learning following sleep deprivation

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The effects of sleep deprivation on the neural substrates of cognition are poorly understood. Here we used functional magnetic resonance imaging to measure the effects of 35 hours of sleep deprivation on cerebral activation during verbal learning in normal young volunteers. On the basis of a previous hypothesis[1], we predicted that the prefrontal cortex (PFC) would be less responsive to cognitive demands following sleep deprivation. Contrary to our expectations, however, the PFC was more responsive after one night of sleep deprivation than after normal sleep. Increased subjective sleepiness in sleep-deprived subjects correlated significantly with activation of the PFC. The temporal lobe was activated after normal sleep but not after sleep deprivation; in contrast, the parietal lobes were not activated after normal sleep but were activated after sleep deprivation. Although sleep deprivation significantly impaired free recall compared with the rested state, better free recall in sleep-deprived subjects was associated with greater parietal lobe activation. These findings show that there are dynamic, compensatory changes in cerebral activation during verbal learning after sleep deprivation and implicate the PFC and parietal lobes in this compensation.

Being deprived of sleep for one night impairs performance on many cognitive tasks[2–6]. Verbal learning is a critical cognitive function whose susceptibility to the detrimental effects of sleep deprivation (SD) has been particularly well replicated[7–9]. Decreases in specific cognitive functions after SD may be associated with impairments in the cerebral systems that form the neural substrates of these functions[1]. In particular, SD has been reported to impair performance on cognitive tasks, including verbal learning tasks, that are putatively dependent upon PFC involvement[4–6]. These observations led us to propose that some cerebral systems, particularly the PFC, would be less activated by cognitive tasks in sleep-deprived than in rested subjects. To test this proposal directly requires the use of noninvasive techniques that measure localized cerebral function. To our knowledge, only four reports using functional brain-imaging methods have described the effects of SD on cognitive performance[10–13]. Although none of these studies investigated the effects of SD on verbal learning, two of them reported reduced cerebral metabolic rate in the PFC following SD[10,12].

We measured localized cerebral activation using the blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) method during performance on a verbal learning task in 13 normal young volunteers after a normal night of sleep (the rested state) and after 34.7 ± 1.2 hours without sleep (the SD state). For each state, the cognitive task alternated between a baseline condition (determining whether a list of nouns was in upper or lower case) and an experimental condition (memorizing a list of nouns)[14]. During task performance, BOLD functional images were acquired at each of 20 sagittally oriented slices covering the whole brain Using high-resolution anatomical images, we identified regions of significant activation during each state separately and regions that were significantly more activated during one state than the other. Significant activation was determined using a cluster threshold method to protect against type I errors[15].

Subjects performed significantly less well on free recall when they were sleep-deprived (4.7 ± 4 words after normal sleep versus 2.8 ± 2 words after SD, $P<0.05$), but showed no significant change in recognition memory (discriminability index, $d' = 2.5 \pm 1$ versus 2.0 ± 1, $P>0.05$). Subjective levels of sleepiness on the Stanford sleepiness scale (SSS)[16] increased (2.3 ± 0.8 versus 4.4 ± 1.4, $P < 0.001$) and levels of concentration decreased (this and subsequent subjective measures used a 5-point Lickert scale; 4.5 ± 0.7 versus 3.5 ± 1.1, $P<0.007$). Subjective estimates of effort (4.0 ± 1.2

Table 1 Regions of significant brain activation following normal sleep and sleep-deprived nights

	Normal sleep			Sleep deprivation	
Brain regions	Talairach Coordinates	Brodmann Areas	Brain regions	Talairach Coordinates	Brodmann Areas
L. MFG	33L, 58A, 6S	10*	L. SFG	18L, 30A, 45S	8
L. MFG/IFG	47L, 24A, 11S	8,9/45,47†‡	L. MFG/OFG	29L, 54A, 4S	10*§
L. OFG	12L, 54A, 14I	10§	L. MFG	52L, 20A, 31S	8/9/46†
L. AC	4L, 31A, 13S	32, 24	L. IFG	53L, 24A, 1I	47‡
L. AC	5L, 21A, 10I	32, 35	L. AC	10L, 24A, 37S	32
L. PMA & SMA	15L, 11A, 61S	6	R. MOG	25R, 92P, 13S	18, 19
	41L, 5A, 25S	6			
	47L, 3A, 42S	6			
	25L, 18A, 53S	6			
L. TP	34L, 4A, 18I	38			
L. MTG	56L, 22P, 5I	21			
L. SOG	43L, 72P, 37S	19			

Each entry represents a significant cluster of activation. Clusters that physically overlap between nights are denoted with identical symbols (*, †, ‡ or §). Premotor area (PMA) and supplementary motor area (SMA) activation after the normal night of sleep included four discrete clusters. The anterior cingulate (AC) gyrus activation after the SD night was slightly dorsal (superior) to that observed after the normal night of sleep. SFG, MFG, IFG and OFG: superior, middle, inferior and orbital frontal gyri, respectively; MTG: middle temporal gyrus; SOG and MOG: superior and middle occipital gyri, respectively. L, left hemisphere; R, right hemisphere. The magnitude of activation of every pixel of each cluster was significant at a minimum *t*-value of 2.18, degrees of freedom 12.