Supplementary Materials for

Ducklings imprint on the relational concept of “same or different”

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Other Supplementary Material for this manuscript includes the following:
(available at www.sciencemag.org/content/353/6296/286/suppl/DC1)

- Table S1
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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, chicks 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, while 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 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 monotonically 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 and the combined shape and colour analysis regardless of which of these three criteria is used. For the colour modality alone, the results remain unchanged.
when preference required five more approaches to one stimulus than the other, becoming non-significant for ten.
Table S1.
Video coding data.

Movie S1
Sample training and testing of two ducklings, one trained on different shapes, the other on same colours. Both successfully discriminate their imprinted concept in testing.
References and Notes


