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Research report

Aposematic colouration enhances memory formation in domestic chicks trained in a weak passive avoidance learning paradigm

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ABSTRACT

The one-trial passive avoidance learning task is commonly used in avian research to explore anatomical, cellular and molecular parameters of learning and memory. Many factors are known to influence the effectiveness and/or duration of such learning events. Combinations of novel odours, such as pyrazine, and aposematic colours, such as brig ht yellow or red, have been shown to induce a long-lasting aversion to food crumbs in 'visual' predators, including birds such as the domestic chick (1). The aim of this study was to (a) examine whether visual complexity played a role in the generation of an aversive response to a novel visual stimulus and (b) to establish whether the duration of memory of an aversive experience could be modified by altering the visual properties of the stimulus. In the first experiment, naïve domestic chicks were trained on a weakly aversive one-trial passive avoidance bead task, in which chicks were allowed to peck at a bead coated with a 10% solution of the bitter-tasting and odorous substance methylanthranilate (MeA). The chicks were trained with (allowed to peck) one of four differently coloured beads dipped in 10% MeA. Chrome, black, yellow or black-and-yellow striped beads were used. 'Recall' of the aversive bead was examined by presenting the (clean) training bead 24 h after training and monitoring avoidance to it compared to a 'neutral' white bead. A high proportion (63%) of chicks trained with the black and yellow striped bead avoided it 24 h after training, whereas little or no avoidance was seen in response to chrome, yellow or black beads. In a second experiment naïve domestic chicks were all trained once only with a black and yellow striped bead coated in a 10% MeA solution, but this time, were tested 24 h later, once only, with either a black, a yellow or a black and yellow striped bead. Nearly 60% of chicks tested with a black and yellow striped bead showed avoidance of the bead, whereas only 23% of those tested with a black bead and 14% tested with a yellow bead showed avoidance. These results confirm the importance of complex warning colouration, when paired with a novel olfactory cue and a bitter taste, in avoidance learning. We conclude that the chicks' response to monochromatic colours (e.g. yellow or black) is not affected by their previous experience with a conspicuously patterned stimulus (yellow and black stripes). Moreover, it suggests a predisposition for chicks to attend to aversive cues associated with 'naturalistic' high contrast colour cue combinations such as black and yellow.

1. Introduction

One-trial passive avoidance learning (PAL) is a robust and widely used task in day-old domestic chicks to explore the anatomical, cellular and molecular processes associated with the consolidation of memory. It is widely used because it enables precise timing of memory formation, it is a simple test to perform and a detailed picture of the biochemical and molecular cascades associated with shorter- and longer-term forms of memory consolidation using this task has been constructed [1,10,18,23,24]. More recently it has been used to delineate other aspects of learning and memory such as memory lability and reconsolidation [1,17].

The standard version of the task involves presenting chicks with a bead coated in a bitter-tasting substance such as methylanthranilate (MeA), at which they spontaneously peck within a few seconds. On pecking, the chicks display a typical disgust response: head-shaking and beak wiping. Chicks subsequently offered beads of similar colours, shapes and smells withhold pecking at such beads, but peck at beads of different colours. The duration of the behavioural response induced by this training procedure varies, depending on the strength of the aversive solution coating the bead [6,11,27] and on subtle variations in training procedures [3–5]. Using what has become known as the Open University (OU) training procedure [3,15], pecking at a bead coated in 100% MeA induces

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avoidance for up to 48 h while pecking at a similar bead coated in 10% MeA induces avoidance for only 6–8 h [13,27].

A number of parameters have been shown to influence the duration of this recall, including hormone status [12,27], bead colour and conspicuousness [3,21,26,28], odours [2,14,16,20] and associated sounds [7,25].

While it has been suggested that warning colouration indicating foul-tasting or dangerous prey, or aposematism, is less likely to be paired with mildly toxic or distasteful prey [30], previous studies suggested that the more conspicuous the visual cue associated with an aversive experience, the more efficacious and long-lasting the retention of the learning experience [21]. We attempted this study as a means of further exploring possible roles of conspicuous visual cues in the consolidation of this task.

2. Methods

2.1. Animals and housing

Fertile eggs from Ross Chunky meat-strain chickens (Maurice Millard Chicks, Trowbridge, U.K.) were incubated for the first 17 days in a communal brooder maintained at 37.5–37.9 °C and on an 8:16 h light/dark cycle. On day 18 of incubation the eggs were transferred to a hatching incubator held at 37–38 °C and maintained on a 12:12 h light/dark cycle and allowed to hatch. The chicks were transferred to a group cage ca. 16–19 h after hatching where they were supplied ad libitum with food and water. Day-old-chicks (24 ± 8 h) of either sex were placed, in pairs, into aluminium pens (20 cm × 25 cm × 20 cm) and supplied with chick starter crumbs. Water was provided from a dish situated at the rear of the pen immediately after training and it was removed 1 h prior to testing. The pens had blue paper-towelling on the floor and were illuminated by an overhead 10-W white pearlescent bulb, which also provided additional heat. One chick in each pair was marked on the back with a spot of animal dye. Each pair of chicks remained undisturbed in their pen for 1 h at 25–28 °C before we trained them.

3. Procedure

The chicks were trained using the Open University procedure as described previously [15]. Briefly, we gave chicks three pretraining trials, each separated by at least 5 min, in which a dry white bead (2.5 mm diameter) on the end of a piece of wire was presented for 10 s. Five minutes later, we presented the chicks with a coloured bead (4 mm diameter) dipped in a 10% solution of methyl anthranilate for 30 s. The bead colours used during training in Experiment 1 included chrome-coloured, black, yellow or a patterned bead consisting of alternating yellow and black stripes (where the stripes are oriented horizontal to wire) [28]. In Experiment 2 all chicks were trained on the black and yellow striped bead. The chrome bead was stainless steel and the other coloured beads were made from hardened yellow (no. 021) and black (no. 025) clay (Cernit porcelain-effect modelling clay, Dreieich, Germany). Black stripes were approximately 0.3 mm wide and there were 6–8 stripes per bead.

Retention, or avoidance of the training bead, was tested 24 h after training. In Experiment 1, testing involved presenting a dry bead, which was the same as that used during training, for 10 s followed 5 min later by a 10-s presentation of a dry white bead. In Experiment 2, chicks were tested with a black, a yellow or a black and yellow striped bead. We excluded a chick from the analysis if it failed to peck during at least two of the three pretraining trials, the training trial or in the second testing trial (overall, 21% of chicks were excluded). In Experiment 1, seven chicks trained and tested on a chrome bead ($n = 15$), three chicks on a black bead ($n = 15$), two chicks on a yellow bead ($n = 14$) and five chicks on the black and yellow striped bead ($n = 19$) were excluded (numbers in parenthesis indicate the sample sizes used in the analysis). In Experiment 2, two chicks tested on a black bead ($n = 17$), three chicks tested on a yellow bead ($n = 15$) and six chicks trained and tested on the black and yellow striped bead ($n = 20$) were excluded.

The number of pecks made at the bead and the number of bouts of headshaking (a rapid lateral movement of the head) were recorded in each trial. We tested each chick once only. A percentage avoidance score for the training bead was calculated as the number of chicks avoiding the bead during the first testing trial that pecked the bead during the second testing trial. We also calculated a mean discrimination ratio by dividing the number of pecks at the bead during the second testing trial by the total number of pecks made during both the first and second testing trials. The avoidance scores were compared with the chi-square test of independence and the mean discrimination ratios, which were normally distributed, were compared with a one-way ANOVA. It is important to note that where chicks peck equally at both beads, they would score a percentage avoidance of zero and a discrimination ratio of 0.5. The remaining behavioural data (number of pecks and headshakes) assumed a non-normal distribution and were thus analysed using Kruskal–Wallis tests [31].

4. Results

4.1. Experiment 1: use of visual cues during training

There was a significant effect of the training stimulus on the avoidance scores ($\chi^2_2 = 11.55$, $P < 0.01$, see Fig. 1a). Chicks trained with the black and yellow striped bead had significantly higher avoidance scores than chicks trained with either a chrome, black or a yellow bead. The same result was found when the discrimination ratios were compared. An independent analysis of variance indicated that there was a significant effect of the training stimulus on the discrimination ratio (ANOVA: $F_{3,62} = 3.90$, $P = 0.01$, see Fig. 1b). Chicks tested with the black and yellow striped bead showed higher

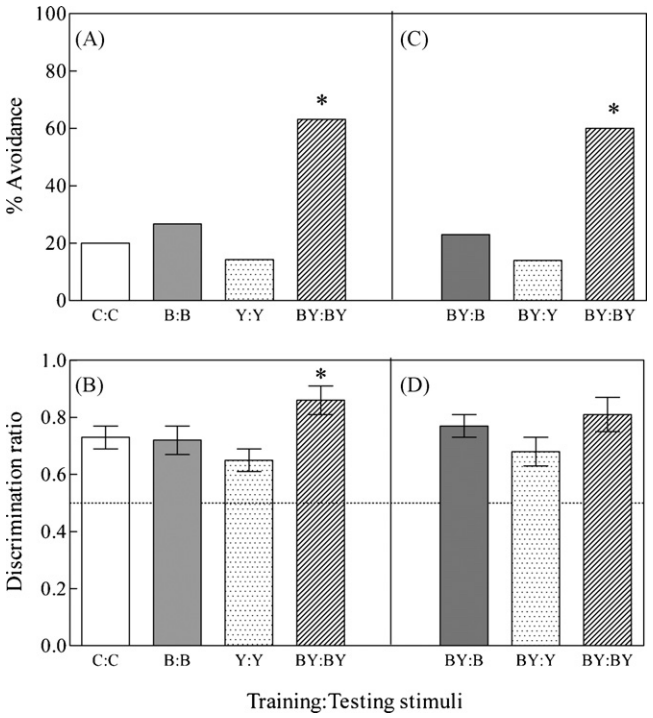

Fig. 1. Effect of training chicks with differently coloured beads on retention of a weak learning task. The data are presented as a percentage avoidance score (A, experiment 1, and C, experiment 2) and mean discrimination ratio (±SEM, B experiment 1 and D, experiment 1 and experiment 2). Letters under each column designate the colour of the training and testing stimuli (B, black; Y, yellow; BY, black and yellow striped). Retention was tested at 24 h. *$P < 0.05$ chi-square test (a), ANOVA followed by post hoc LSD test (b), $n = 15$–19 per group.