

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

ABSTRACT

The one-trial passive avoidance learning (PAL) task is commonly used in avian research to explore various parameters of learning and memory. Many factors, including combinations of novel odours and aposematic colours, influence the effectiveness and/or duration of such learning events. This study explored whether visual complexity influenced formation of an aversive response to a novel visual stimulus and whether memory duration could be modified by altering visual properties of the stimulus. Naïve chicks were trained using a weakly aversive PAL task, in which chicks peck at a chrome, black, yellow or black-and-yellow-striped (BYS) bead coated with a 10% solution of methyl-anthranilate (MeA). Chicks were offered the same (clean) bead during testing 24h later. Experiment one showed a higher proportion of chicks trained with the BYS bead avoided it 24h after training. In a second experiment all chicks were trained with a BYS bead coated in a 10% MeA solution, and tested 24h later, with either a black, a yellow or a BYS bead. Nearly 70% of these chicks showed avoidance of the BYS bead, whereas only around 30% of those tested with a black bead or 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 (BYS). 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.

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 avoidance for up to 48 hours while pecking at a similar bead coated in 10% MeA induces avoidance for only 6-8 hours [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.

METHODS

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 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 × 25 × 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.

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 0.3mm 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, 7 chicks trained and tested on a chrome bead (n=15), 3 chicks on a black bead (n=15), 2 chicks on a yellow bead (n=14) and 5 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, 2 chicks tested on a black bead (n=17), 3 chicks tested on a yellow bead (n=15) and 6 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 percent 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].

RESULTS

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 levels of avoidance compared to chicks tested with a chrome, yellow or black bead showed. The mean discrimination ratios for each group of chicks were all significantly greater than 0.5 ($P<0.05$).

Table 1 shows the pecking and headshaking data for chicks trained with each type of bead. There were significant differences during training and testing: birds trained with a black and yellow striped bead pecked less than birds trained with a chrome, black or a yellow bead. There were no differences in the raw scores for pretraining or pecking at the white bead during testing. Thus the behaviour of the chicks was not biased prior to the experiment or by the experimental procedure alone.

Bouts of headshaking were evoked most strongly during training but this was not affected by bead colour. High levels of head shaking were still evident in test 1 by chicks tested with a black bead or those presented with a black and yellow striped bead ($P<0.05$, see Table 1) but there were no difference between groups during test 2.

Experiment 2: Use of visual cues during testing.

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 70% of chicks tested with a black and yellow striped bead showed avoidance of the bead, whereas only 33% of those tested with a black bead and 29% tested with a yellow bead showed avoidance.

Table 2 shows the pecking and headshaking data for chicks trained with each type of bead. Birds trained with a black and yellow striped bead pecked less than birds trained with a black or a yellow bead at test, and this effect was significant ($P < 0.05$). There were no differences in the raw scores for pretraining or pecking at the white bead during testing. Thus the behaviour of the chicks was not biased prior to the experiment or by the experimental procedure alone. There was no significant effect of the training stimulus on the mean discrimination ratio and the mean discrimination ratios for each group of chicks were all significantly greater than 0.5 ($P < 0.05$)

Bouts of headshaking were evoked most strongly during training but this was not affected by bead colour. High levels of head shaking were still evident in test 1 by chicks tested with a black and yellow striped bead ($P < 0.05$, see Table 2) but there were no difference between groups during test 2.

DISCUSSION

The results of these experiments indicate that it is not simply colour novelty alone or in combination with specific odorants which influence the duration of recall of the one-trial passive avoidance learning task [20,21,25]. Complex patterned colouration, such as that of black and yellow striped beads, appears to enhance the duration and efficacy of recall in this task. The enhanced recall of the task is specific to the patterned beads and not transferred to beads of the composite colours. Chicks trained using the black and yellow bead showed reduced pecking at that bead, prior to any previous experience with such patterned colouration, potentially indicating an innate bias against objects with aposematic colouration [28,29]. At test, chicks clearly recalled this complex colouration more effectively than the monochromatic components of the bead colour, showing enhanced recall and discrimination at test at 24 hrs.

These studies confirm previous results suggesting that the conspicuous prey items may enhance attack behaviour but also, where the item is distasteful, increase the strength of avoidance learning [22]. This would seem to be enhanced by the novelty of the item pecked, such as the unusually patterned bead [19,22].

Prey object colour preferences may be encoded at a subtelecephalic level in chicks, particularly when they are exposed to light during rearing [32]. The current study suggests that prey colour preference, or appetitively driven learning preferences may also rely on higher learning centres in the chick brain, or at least are significantly influenced by exposure to experiences known to activate regions in the telencephalic areas of the brain. Reduced pecking at an aposematically coloured bead during training fits with a concept of innate avoidance of such colour patterns. However chicks did peck at these beads during training, again reflecting results of previous studies showing that automimics (copying of aposematism to avoid predation) is often only partially

effective as chicks will peck (albeit at a reduced level) at more highly avoided objects as a form of ongoing sampling behaviour [8].

There was no significant difference in the size of the various training beads so that unconditioned aversion (shown by reduced pecking at the black and yellow bead during training) and conditioned aversion (shown by enhanced recall following the ‘weakly’ aversive training protocol, [27] was unlikely to have arisen solely because of increasing prey conspicuousness [9]. However, previous, very stable, avoidance of yellow-coloured food objects has been reported in chicks and is particularly pronounced when the yellow colouration is coupled with a novel odour [25]. Thus, the significantly enhanced recall of objects with warning colouration, novel odour and aversive taste is perhaps to be expected. While this may form an instance of classical conditioning evident in the chick [21] it is clear that the salience of the visually conspicuous object can and does influence the salience of the learning experience (see [30] – in that training with the black and yellow bead enhanced the duration of recall unlike training with either a yellow or black bead. It is also clear that it is the combination of the two colours which is most pertinent, as training with the black and yellow bead did not produce a generalized response to black or yellow alone. Moreover, the combination of a novel coloured bead, with a novel odour and an aversive experience was insufficient to enhance recall. Hence, at least within the limited confines of the colour ranges tested here, simple odour and colour novelty alone [20] do not trigger enhanced salience and thus recall.

Table 1. Behaviour of chicks during bead presentations in Experiment 1

Training	Stimulus
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	Chrome	Black	Yellow	B/Y Stripes	Test statistic
Pecking					
Pretraining	3.91 ± 0.60	2.87 ± 0.43	2.67 ± 0.45	2.51 ± 0.42	4.60
Training	2.33 ± 0.29 ^b	2.00 ± 0.22 ^b	2.71 ± 0.38 ^b	1.21 ± 0.10 ^a	17.94*
Test 1	1.67 ± 0.30 ^b	1.40 ± 0.31 ^b	2.43 ± 0.42 ^b	0.58 ± 0.19 ^a	15.60*
Test 2	3.73 ± 0.32	2.93 ± 0.37	3.93 ± 0.60	2.74 ± 0.31	5.61
Headshake					
Pretraining	0.13 ± 0.06	0.22 ± 0.08	0.14 ± 0.07	0.16 ± 0.05	0.81
Training	10.33 ± 0.79	10.40 ± 0.72	10.21 ± 0.77	10.53 ± 0.73	0.16
Test 1	0.27 ± 0.27 ^a	0.80 ± 0.30 ^b	0.21 ± 0.15 ^a	0.89 ± 0.25 ^b	8.26*
Test 2	0.13 ± 0.09	0.00 ± 0.00	0.14 ± 0.10	0.11 ± 0.07	2.18

Values are means ± SEM. * $P < 0.05$, Kruskal-Wallis H test, $df=3$ (H values given). Data with different superscript letters are significantly different from each other ($P < 0.05$).

Table 2. Behaviour of chicks during bead presentations in Experiment 2

	Testing Stimulus			Test statistic
	Black	Yellow	B/Y Stripes	
Pecking				
Pretraining	3.04 ± 0.41	3.45 ± 0.53	2.50 ± 0.39	2.48
Training	1.50 ± 0.17 ^a	2.21 ± 0.33 ^b	1.25 ± 0.10 ^a	7.64*
Test 1	1.28 ± 0.33 ^b	2.21 ± 0.53 ^b	0.65 ± 0.20 ^a	8.21*
Test 2	3.11 ± 0.63	4.00 ± 0.82	2.15 ± 0.25	5.00
Headshake				
Pretraining	0.11 ± 0.04	0.21 ± 0.08	0.18 ± 0.06	1.05
Training	10.67 ± 0.63	10.36 ± 0.86	10.10 ± 0.67	0.35
Test 1	0.39 ± 0.18 ^a	0.14 ± 0.14 ^a	1.05 ± 0.27 ^b	7.85*
Test 2	0.00 ± 0.00	0.07 ± 0.07	0.10 ± 0.07	1.77

Values are means ± SEM. * $P < 0.05$, Kruskal-Wallis H test, $df=3$ (H values given). Data with different superscript letters are significantly different from each other ($P < 0.05$).

Figure 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,C) and mean discrimination ratio (+ SEM, B,D). 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.

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