

Performance on a categorisation task suggests that removal of environmental enrichment induces 'pessimism' in captive European starlings (*Sturnus vulgaris*)

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Abstract

Improving the quality of life of captive animals is dependent on developing valid measures of how animals feel about their lives. It has recently been suggested that biases in information processing may offer a novel means of understanding animal emotions. Anxious and depressed people tend to interpret ambiguous information negatively. We explored the proposal that such cognitive biases also exist in non-human animals and could therefore be used as novel measures of animal welfare. We used a novel cognitive bias task based on a learnt taste aversion to determine whether birds deprived of environmental enrichment show biases in their classification of ambiguous signals. We hypothesised that starlings in enriched cages should be more likely to classify ambiguous signals as being associated with a positive outcome than starlings housed in standard, unenriched cages. Starlings were trained on a go/no-go procedure to discriminate between two visual stimuli (cardboard lids of white and dark grey) associated with outcomes of a different value (palatable and unpalatable mealworms hidden underneath). Individual birds' responses to unreinforced, intermediate stimuli (various shades of grey between white and dark grey) were subsequently examined while each bird was housed sequentially in both standard and enriched cages. The probability of a bird classifying an ambiguous pale grey lid as hiding a palatable mealworm was lower in standard cages than enriched cages, but this difference was found only in birds that received enriched cages first. Our results can be interpreted as showing a pessimistic bias in birds that have recently experienced a decline in environmental quality. These findings support the use of cognitive bias-based tasks as a novel, non-invasive technique for assessing welfare in non-human animals.

Keywords: affective state, animal welfare, cognitive bias, contrast effect, emotion, environmental enrichment

Introduction

It is widely accepted that we should strive to provide the animals in our care with a good quality of life (QoL). This entails both producing a definition of what we mean by QoL, and developing scientific methods for measuring QoL. In the assessment of human QoL verbal self-report is an important component, but unfortunately animals cannot tell us directly how they feel about their lives. To assess animal emotions we are forced to rely on indirect methods such as measurement of stress hormones or abnormal behaviour patterns. Recently, Mendl and Paul (Mendl & Paul 2004; Paul *et al* 2005) proposed a novel approach to assessing affective state in animals, based on measuring emotionally induced biases in decision-making. Their approach centres on the assumption that in both humans and other animals emotional states share a common evolved function. In humans, it is well established that emotional states cause adaptive biases in cognition. For example, anxious people are more likely to assume a negative interpretation of an ambiguous sentence such as "The doctor examined little Emily's growth" (Eysenck *et al* 1991). In general, negative affective states such as anxiety and depression are associated with a more

pessimistic interpretation of ambiguous stimuli. This finding makes evolutionary sense: if an organism has information that the world is currently a dangerous place, it is adaptive to adopt a conservative threshold for responding to potentially threatening stimuli.

In a first attempt to explore whether animals show similar environmentally induced biases in decision-making, Harding *et al* (2004) devised a task in which rats were required to categorise an ambiguous signal as either positive or negative. The animals were trained on an operant go/no-go task to press a lever to obtain a food reward on hearing a tone of one frequency, but to refrain from pressing the lever to avoid unpleasant white noise on hearing a tone of a different frequency. Once the rats had acquired this discrimination they were presented with unreinforced, ambiguous, intermediate-frequency tones. Rats housed in unpredictable conditions known to induce symptoms of depression showed fewer and slower responses to these ambiguous tones than animals housed in control conditions. Thus, the depressed rats showed reduced anticipation of a positive event similar to pessimistic cognitive biases seen in depressed and anxious humans.

The aims of the current study were to extend the work of Harding *et al* (2004) by developing a novel cognitive bias task that is quicker to train than their operant task, and applying it to a new species, the European starling (*Sturnus vulgaris*) undergoing a different environmental manipulation also designed to produce differences in affective state. The task we developed was based on a conditioned taste aversion. Such associations have the advantage of being acquired quickly, typically within a single trial, and are also more resistant to extinction than learning based on positive reinforcement, thus allowing the inclusion of a greater proportion of unreinforced probe trials. We trained the aversion using a simple, naturalistic foraging task that can be implemented in the subjects' home cages, thus removing the need to catch them and move them to operant chambers. We used our task to measure cognitive biases in starlings housed in standard versus enriched cages. Environmental enrichment is widely believed to have beneficial consequences for animal welfare. Evidence for welfare benefits comes from a number of different sources including behaviour, physiology and neurobiology. For example, animals exposed to enriched environments show reduction in abnormal and injurious behaviour patterns, reduction in plasma cortisol levels and increases in brain weight and neuron density (reviewed in Young 2003). As in both the human and rat tasks, we probed the animals with ambiguous cues intermediate between those used in conditioning, because there is evidence that emotionally induced cognitive biases may be especially evident when subjects are required to interpret ambiguous information (Bower & Forgas 2000). We tested the hypothesis that if environmental enrichment is associated with a more positive affective state, this will be reflected in less pessimistic responses to the ambiguous cues when the birds are housed in enriched cages.

Materials and methods

Animals

Six wild-caught European starlings (*Sturnus vulgaris*), three males and three females, were housed individually in cages 75 × 45 × 44 cm in a room with a 14:10 h light:dark cycle. Initially each cage was equipped with two dowel perches and two water bottles. The birds had *ad libitum* Purina wild game starter and fruit available except during the daily experimental sessions and for 30 min prior to this. Additionally, the birds received 4–16 mealworms (*Tenebrio larvae*), a preferred prey type, during the experimental sessions. Experimental sessions took place in the home cages.

Starlings were caught from the wild under licence from English Nature. Our research adhered to the Association for the Study of Animal Behaviour Guidelines for the Use of Animals in Research. Starlings were released to the wild following the completion of our studies.

Cognitive bias task

For the foraging task we used a 3.5 cm Petri dish mounted on a white ceramic tile and covered with a 5 cm diameter cardboard lid. The birds were trained to flip the lid off the

dish to obtain a mealworm hidden underneath (see Barnett *et al* 2007 for a similar task). A session comprised 16 trials in which the dish was placed in the starling's cage and the bird was given 3 min in which to decide whether to flip the lid and consume the mealworm, after which time the dish was removed.

The birds were trained on a visual discrimination: palatable mealworms (injected with 0.2 ml water) were associated with white lids, whereas unpalatable mealworms (injected with 0.2 ml 2% quinine sulphate solution) were associated with dark grey lids (80% greyscale). Sessions consisted of a total of 16 trials: eight with white lids, and eight with dark grey lids. In order to avoid sequences of more than four trials of one type, the session was divided into four blocks of four trials. Each block was constrained to contain two trials of each type, but the order in which these four trials occurred was random. The birds were assumed to have learnt the discrimination when they had shown a significant difference (binomial test, $P < 0.05$) in the proportion of white and dark grey lids flipped for three consecutive sessions.

Finally, unreinforced probe trials were inserted into the sessions that now comprised eight reinforced trials (four white and four dark) and 20 probe trials (four each of white, 20%, 40%, 60% and 80% grey). As above, the session was divided into four blocks of seven trials with each block containing one trial of each type. The order of trials within each block was random. The behavioural variable of interest was the probability that the bird flipped the lid in the probe trials.

Housing manipulation

We compared the probability of lid flipping in the probe trials when the birds were housed in two cage types. Standard cages were identical to the cages used during training. Enriched cages were the same size and shape as standard cages but had natural branches of differing thickness placed at different heights and angles, water baths (20 cm diameter) and a plastic tray of bark chippings (25 × 35 cm) in which the birds could probe. Four birds received an enriched cage followed by a standard cage, and the remaining two birds received the treatments in the opposite order. At the start of the housing manipulation all birds were transferred to new cages (either standard or enriched). The birds were given two days to settle in their new cages followed by five days of experimental sessions. Following their first cage treatment all birds were transferred into their other cage treatment for a further seven days. The second week proceeded identically to the first with the exception that the first experimental session had to be aborted for reasons beyond our control resulting in just four days of experimental sessions.

Data analysis

The experimental design yielded a maximum of 20 probe trials from a single experimental session: four trials at each of five possible lid shades. Thus, overall the data comprised a total of 20 probes at each lid shade in the first cage treatment and 16 probes (because of the aborted first

experimental session) at each lid shade in the second treatment. For each lid shade in each treatment we computed the proportion of lids that were flipped by a bird. Data were analysed using repeated-measures general linear model on the arcsine square-root-transformed proportions. Factors included in the model were cage type (within subjects, two levels) and treatment order (between subjects, two levels). We assumed a criterion for significance of $P < 0.05$.

Results

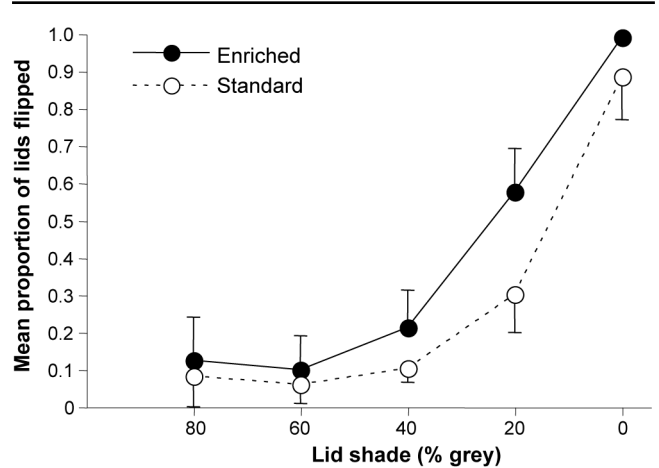
Data from the probe trials with the two trained shades (ie 0% and 80% grey) showed that the birds learned the visual discrimination: the proportion of trials on which subjects flipped the 80% grey lids was 0.106 ± 0.101 (mean \pm 1 standard error), whereas the proportion of trials on which subjects flipped the 0% lids was 0.944 ± 0.056 . When confronted with lids of the untrained intermediate shades, the birds treated the two darker shades (40% and 60%) similarly to the 80% lids, flipping them in 0.167 ± 0.065 and 0.083 ± 0.072 of trials, respectively; however, the birds were ambivalent about the 20% grey lids, flipping them on approximately half (0.458 ± 0.086) of trials (Figure 1). The general linear model conducted on the proportion of 20% grey lids flipped showed a non-significant trend of cage type ($F_{1,4} = 6.040$, $P = 0.070$) and a significant cage \times order interaction ($F_{1,4} = 11.530$, $P = 0.027$). The interaction reflected the fact that the proportion of lids flipped was lower in the standard cages than in the enriched cages, but only when the birds had experienced the enriched cages prior to being transferred to the standard cages (Figure 2).

Discussion

The response of starlings to an ambiguous visual cue is affected by their recent experience of environmental enrichment. Starlings trained to approach and flip white lids to obtain palatable mealworms, but to avoid dark lids associated with unpalatable mealworms, were less likely to approach and flip intermediate pale grey lids when they had recently been moved from an enriched to a standard cage than when they had experienced the cages in the opposite order. We interpret these data as showing a pessimistic interpretive bias in birds that had experienced a recent decline in their environmental quality. We suggest that this bias is indicative of a more negative affective state in birds moved from enriched to standard cages than in birds experiencing the cage types in the opposite order.

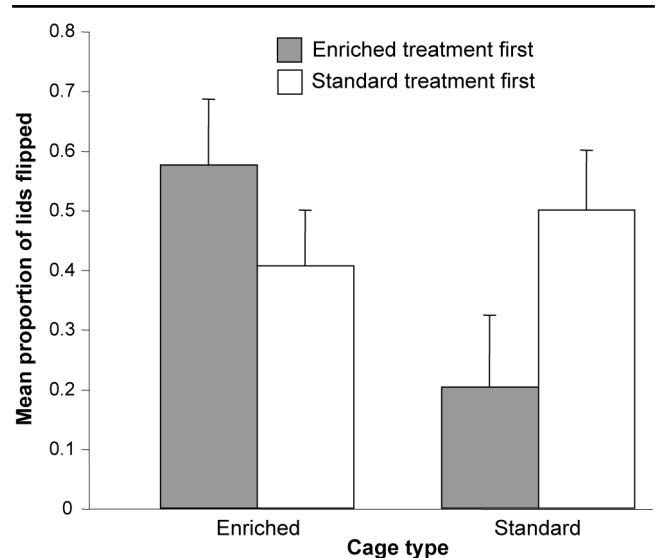
Our finding that environmental enrichment can affect measures of pessimism fits with other types of data from a host of species suggesting that welfare is improved in enriched environments (Young 2003). For example, we have previously found that starlings housed for one week with the same enrichments used in the current study showed fewer repetitive behaviour patterns and lower corticosterone levels than starlings housed in standard, unenriched cages (Asher & Bateson, unpublished data 2007). Therefore, our current results support the use of cognitive bias tasks as a novel, non-invasive technique for assessing affective state in animals.

Figure 1



The mean (\pm 1 standard error) proportion of lids flipped for each of the five types of probe trial in enriched and standard cages. The birds flipped a lower proportion of the ambiguous 20% grey lids when they were housed in standard cages, possibly indicative of greater pessimism that the mealworm obtained would be palatable.

Figure 2



The mean (\pm 1 standard error) proportion of 20% grey lids flipped in the enriched and standard treatments divided according to the order in which the birds received these treatments. The graph shows an interaction between the cage treatment and the order in which this was experienced. The birds flipped a lower proportion of ambiguous 20% grey lids in standard cages than enriched cages, but only when they had previously experienced enriched cages.

One aspect of our data that we did not predict was the effect of the order in which the bird received the two cage treatments. We believe that this may be the first study to show that the affective response to a given level of environmental enrichment depends on the prior experience of the animal. However, our finding of an asymmetrical response to losses and gains fits with findings from a number of other domains, such as negative contrast effects in the animal conditioning literature (eg Papini *et al* 2001) and the endowment effect in humans (Kahnemann *et al* 1990).

Given our evidence that cognitive bias can provide a valid measure of affective state, is there any reason to favour this approach over other currently available measures of welfare? We believe that an argument for using cognitive bias can be made if it provides information that other measures do not. Two justifications have been proposed. First, it is possible that cognitive bias may be better than some measures at assessing the valence (pleasantness *versus* unpleasantness) of an emotion as opposed to just the level of arousal (Paul *et al* 2005). Second, in humans, the cognitive biases associated with emotional states may arise directly as a result of conscious emotional feelings entering into the processes of judgement and decision-making (Schwarz & Clore 1996; Bower & Forgas 2000). If this is correct, then it suggests that if similar biases are identified in animals this may be indicative of the presence of conscious emotional experience in these species. If cognitive bias does provide different information from other measures of welfare then we predict that there should be situations in which dissociations between the different measures occur. It is intriguing to speculate whether the order effect observed in the current study is a possible example.

To be practically useful as a measure of how animals feel, cognitive bias needs to be easy to measure in applied settings. We believe that the task developed for this study offers significant advantages in this respect over operant cognitive bias tasks previously used in rats and starlings (Harding *et al* 2004; Matheson *et al* 2007). By tapping into the taxonomically widespread tendency that animals have to avoid foods that have previously made them nauseous, we have found a task that is both simple to implement and fast to train, and that is likely to work in a wide range of species including chickens and laboratory rodents.

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