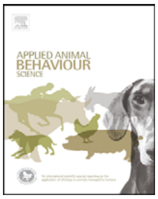
Applied Animal Behaviour Science 118 (2009) 182–190

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim



Can we use starlings’ aversion to eyespots as the basis for a novel ‘cognitive bias’ task?[☆]

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ARTICLE INFO

Article history:
Available online 17 March 2009

Keywords:
Cognitive bias
Eyespots
Alarm calls
White noise
Anxiety

ABSTRACT

Experiments in humans have shown that changes in emotional (affective) state cause adaptive changes in the processing of incoming information, termed “cognitive bias”. For instance, the states of anxiety and depression have been shown to be associated with “pessimistic” judgements of ambiguous stimuli intermediate between stimuli associated with positive and negative outcomes. This phenomenon provides a promising method for objectively assessing animal emotional states and has been successfully demonstrated in preliminary studies. However, the experiments yielding these results required extensive training to establish the necessary positive and negative associations. Here we present an experiment using responses to eyespot stimuli that are naturally aversive to many bird species, and require no explicit associative training. We manipulated the state of wild-caught European starlings (*Sturnus vulgaris*) by playing one of four possible sounds: starling “threat call” (control manipulation), a sparrowhawk call (i.e. predator), starling alarm call or white noise, on the assumption that the latter three sounds would cause anxiety. Immediately following the auditory stimulus, we recorded the birds’ behaviour in the presence of each of three visual stimuli: eyespots, ambiguous eyespots or no eyespots. We hypothesised that there would be an interaction between the state of the birds and their response to eyespots, with birds showing enhanced aversion to ambiguous eyespots when anxious. We found evidence that white noise and alarm calls generated anxiety, and that eyespots were aversive. However, there was no interaction between state and response to eyespots. In an attempt to understand our failure to obtain the predicted cognitive bias, we discuss evidence that the aversive nature of eyespots is not attributable to predator mimicry, and is therefore not modulated by anxiety.

1. Introduction

Objectively assessing the affective (i.e. emotional) state of animals is one of the primary concerns of welfare science. A promising recent approach focuses on assessing how emotional processes affect cognitive function (Mendl and Paul, 2004; Paul et al., 2005). The underlying theoretical background was initially derived from work in humans, where differences in “trait” (stable variability between individuals) and “state” (transitory variability within individuals) anxiety are associated with well-defined biases in performance on cognitive tasks that test attention, memory and judgement (reviewed by Paul et al., 2005). For example, this literature suggests that individuals suffering from negative affective states associated with anxiety and depression are more likely to interpret ambiguous stimuli as threatening, or as indicative of negative outcomes (e.g. Eysenck et al., 1991). These “pessimistic” cognitive biases make sense from an evolutionary perspective under the assumption that negative

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[☆] This paper is part of a special issue entitled “Animal Suffering and Welfare”, Guest Edited by Hanno Würbel

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0168-1591/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.applanim.2009.02.015

affective states are an adaptive response to receiving information that there may be threats present in the environment. Under these circumstances it is adaptive to adopt a more conservative criterion for classifying an ambiguous event as a likely threat (Haselton and Nettle, 2006). To avoid charges of anthropomorphism, we define "pessimism" operationally as an increased probability of classifying an ambiguous stimulus as predicting a negative outcome.

In an attempt to explore whether animals show similar "pessimistic" cognitive biases, Harding et al. (2004) trained rats to press a lever for food reward on hearing a positive 2 Hz tone but to refrain from lever pressing to avoid punishment with white noise on hearing a negative 4 Hz tone. Once trained, rats were then tested with ambiguous intermediate tones (2.5, 3.0 and 3.5 Hz). Rats kept in "unpredictable" housing conditions known to induce symptoms of depression were less likely to lever press in response to the intermediate tones. This result was interpreted as evidence for a "pessimistic" cognitive bias in rats in a negative affective state. Analogous experiments on European starlings (*Sturnus vulgaris*) have provided support for this result, by showing that birds housed in smaller cages lacking environmental enrichment are also more likely to classify an ambiguous stimulus as predicting a more negative outcome (Bateson and Matheson, 2007; Matheson et al., 2008). The design of the tasks used in the above experiments is summarised in Table 1.

In all of the above experiments, the demonstration of cognitive bias relies on the establishment of a continuous or discrete stimulus scale with positive reinforcement associated with one end and negative (or less positive) reinforcement with the other. Extensive training of experimental subjects was required in order for them to learn the necessary positive and negative associations (Harding et al., 2004; Bateson and Matheson, 2007; Matheson et al., 2008; Burman et al., 2008). There are a number of drawbacks associated with this extended training. First, it is very time-consuming and hence may be difficult to transfer to situations where a practical assessment of animal welfare is needed rapidly and cost-effectively. Second, extensive training introduces potential experimental error whereby subjects perceive and learn about additional elements that were not foreseen by the experimenter, e.g. side-biases (Jackson et al., 1998); "superstitious" responses (Doughty et al., 2001); or interactions between the stimuli and reinforcers (Matheson et al., 2008). Finally, a carefully controlled training regime is only possible where all individuals are currently experiencing the same conditions (as those in experimental conditions are). However, in non-experimental circumstances differences in environmental conditions and prior affective state can occur. These in turn are known to lead to changes in the neuronal processes underlying learning and memory (LeDoux, 1992; McEwen and Sapolsky, 1995) that could impact on the findings of a cognitive bias trial.

Our aim in the current paper is to address the above drawbacks of previous cognitive bias tasks by exploiting stimuli that animals find naturally aversive, meaning that no training is required to establish the association between a stimulus and a negative outcome. The eyespot stimuli used by many lepidoperan species to deter bird attacks are a good potential candidate for use in experiments with European starlings. Eyespots are known to be aversive to passerines, and are effective in preventing birds from feeding on both live lepidopterans and paper models (Vallin et al., 2005; Stevens et al., 2007). Though the mechanism for the aversive effect of eyespots is unknown, one theory is that they mimic the eyes of the natural predators of small passerines (mammals and raptors; for a review see Stevens (2005)). In support of this theory, an extensive set of laboratory experiments showed that eyespots enclosed within a head shape (designed to resemble an owl), and displayed adjacent to a feeder, were particularly effective at deterring starlings from feeding (Inglis et al., 1983). These results imply that the negative outcome associated with eyespots could be predation.

On the basis of these findings we chose to use eyespots adjacent to food as our negative stimulus. We used a similarly sized visual stimulus, but with no eyespots, adjacent to food as our positive stimulus. As our intermediate test stimulus we added visual noise to the eyespot stimuli (see Section 2.3 for details) on the grounds that eyespots with reduced contrast have been shown to produce a deterrent effect of reduced magnitude (Stevens et al., 2007).

In order to observe a cognitive bias, it is necessary for the affective state of the experimental subjects to interact with their response to the ambiguous predictors of food reward. In previous experiments experimental manipulations of state have involved changes in housing conditions that are theorised to cause an anxious and/or depressed state (e.g. Harding et al., 2004; Bateson and Matheson, 2007; Burman et al., 2008). The success of these experiments relies on

Table 1
Methodology of previous cognitive bias tasks.

Species	Stimuli		Outcomes		State manipulation	Reference
	Positive	Negative	Positive	Negative		
Rat	2 Hz tone	4 Hz tone	45 mg food pellet	30 s 70 dB white noise	Predictability of housing	Harding et al. (2004)
Rat	Location of food bowl in test arena	Alternative location of food bowl	45 mg food pellet	No food reward	Housing enrichment	Burman et al. (2008)
Starling	10 s light	2 s light	Instant food: 45 mg pellet	15-s delayed food: 45 mg pellet	Cage size and enrichment	Matheson et al. (2008)
Starling	White lid	80% grey lid	Palatable mealworm	Unpalatable quinine-injected mealworm	Cage enrichment	Bateson and Matheson (2007)