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The role of differential reinforcement in predator avoidance learning

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Abstract

Little is known about how predator recognition develops under natural conditions. Predispositions to respond to some stimuli preferentially are likely to interact with the effects of experience. Convergent evidence from several studies suggests that predator-naïve tammar wallabies (*Macropus eugenii*) have some ability to respond to vertebrate predators differently from non-predators and that antipredator responses can be selectively enhanced by experience. Here, we examined the effects of differential reinforcement on responses to a model fox (*Vulpes vulpes*), cat (*Felis catus*) and conspecific wallaby. During training, tammars experienced paired presentations of a model fox and a simulated capture, as well as presentations of a wallaby and a cat alone. Training enhanced responses to the fox, relative to the conspecific wallaby, but acquired responses to the two predators did not differ, despite repeated, non-reinforced presentations of the cat. Results suggest that experience interacts with the wallabies' ability to perceive predators as a natural category.
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1. Introduction

It is crucial for animals to discriminate between events of different functional significance so that they can respond adaptively. The question of how animals recognise natural stimuli has been primarily addressed using model systems such as imprinting (Johnson and Morton, 1991) and song learning (Marler, 1997). Much less is known about how prey visually discriminate predators from other, morphologically similar, innocuous stimuli. There is some evidence that specific cues, such as frontally-placed eyes, trigger fear responses in predator-naïve individuals from a broad range of species (fish: Coss, 1979; Karplus and Algom, 1981; Altbäcker and Csányi, 1990; Miklósi et al., 1995; reptiles: Hennig, 1977; Burghardt and Greene, 1988; birds: Scaife, 1976; Curio, 1993; mammals: Coss, 1978; Topál and Csányi, 1994).

Predator recognition may also develop as a consequence of experience and several learning mechanisms have been identified (Schleidt, 1961a,b; Csányi, 1985; Tulley and Huntingford, 1987; Magurran, 1989, 1990; Seyfarth and Cheney, 1986; Huntingford and Wright, 1992; Curio, 1993; Palleroni, 1999). With the exception of frequency-dependent learning (Schleidt, 1961a,b), all of these processes involve an interaction between predispositions to respond to some stimuli preferentially, perhaps those that trigger antipredator

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behaviour in naïve animals, and the effects of experience. For example, the initial presentation of a model Australian honeyeater (*Philemon corniculatus*) elicits a greater response from blackbirds (*Turdus merula*) than a plastic bottle. When each of these stimuli is subsequently paired with the experience of a conspecific apparently mobbing them, the magnitude of the acquired antipredator response to the honeyeater is much greater (Curio, 1993).

There is good evidence that social stimuli, such as conspecific alarm signals or fear responses, can facilitate predator avoidance learning (Curio, 1988; Mineka and Cook, 1988; Magurran, 1989). Individual experiences are likely to have a similar effect. The aversive consequences associated with predatory events, such as being chased, attacked and perhaps injured, are likely to enhance antipredator responses, so long as the potential prey survives. Conversely, responses to non-predators should decrease as a consequence of harmless encounters. Individual experience thus has the potential to enhance the specificity of pre-existing antipredator responses.

Prey also encounter predators that are not hunting. Under these circumstances, there will be no pairing with aversive events, but it would be maladaptive for fear responses to decrease as a consequence. There is some evidence that differential habituation to predators and harmless species maintains heightened responses, despite equal numbers of harmless encounters with both kinds of stimuli (see Curio, 1993, for a review). For example, responses of paradise fish (*Macropodus opercularis*) to goldfish (*Carassius auratus*) decrease more rapidly over the course of repeated presentations than their responses to predatory pike (Csányi, 1985).

Antipredator responses might also be maintained if animals have a strong tendency to generalise the effects of their experiences with one predator to other, morphologically similar, predators. Such response generalisation would only confer a functional benefit if it was sufficiently robust to resist being counteracted by habituation. To our knowledge, the combined effects of generalisation and habituation on antipredator responses have not been explored empirically.

Discrimination training involving aversive reinforcement of predator models and non-reinforcement of non-predator models is likely to simulate the consequences of naturally occurring encounters. This technique can hence be used to study, under controlled conditions, the way in which individual experience with threatening and innocuous stimuli produces selective learning.

Recent work on predator recognition in tammar wallabies (*Macropus eugenii*), a medium-sized Australian macropod, has shown that predator-naïve tammars may perceive predators as different from other quadrupedal vertebrates (Blumstein et al., 2000; Griffin et al., 2002). We have successfully enhanced the responses of predator-naïve tammars to a taxidermically-prepared model predator (fox, *Vulpes vulpes*) by presenting this stimulus in conjunction with an aversive event (a human simulating a capture attempt, Griffin et al., 2001). After training, the animals generalised their acquired response to a morphologically similar predator (cat, *Felis catus*), but not to a size-matched non-predator (goat, *Capra hircus*). However, tammars also showed a transient increase in response to a conspecific wallaby, even though this model had never been paired with simulated capture.

In the present study, we examined whether differential reinforcement of the fox and wallaby would enhance the specificity of the acquired antipredator response. We also tested whether non-reinforcement had a differential effect on responses to a predatory stimulus (cat) and a non-predatory one (wallaby). During training, we conducted four paired presentations of a model fox and simulated capture. These trials were identical to those used in our earlier study (Griffin et al., 2001). The training protocol differed from that used previously in that subjects also received four non-reinforced presentations of a conspecific wallaby and four non-reinforced presentations of a cat. To assess the effects of training, we measured responses to all stimuli both before training (*pre-training trials*) and afterward (*post-training trials*). Our design incorporated a control group that was matched for total exposure to all stimuli, but without any predictive relationship between fox and simulated capture. Comparisons between experimental and control groups hence enabled us to detect changes in behaviour specifically attributable to associative learning. We expected that selective reinforcement of the fox would inculcate a differential response to the predator and to the wallaby. In contrast, we had no a priori basis for predicting how training would affect responses to the cat because we did not know what the balance would be between gen-

eralisation and habituation. The present study allowed us to test whether non-reinforced presentations of the felid would be sufficient to abolish the generalisation of acquired antipredator responses from fox to cat documented in earlier experiments (Griffin et al., 2001).

2. Materials and methods

2.1. Subjects and husbandry

We randomly selected 16 adult tammar wallabies of approximately 2–5 years of age (8 females and 8 males). Animals were temporarily removed from large breeding groups in the Macquarie University Fauna Park and returned to these at the end of testing. All individuals descended from a Kangaroo Island population which has been not been exposed to mammalian predators since the isolation of the island from mainland Australia at the end of the last glaciation, 9500 years ago (Blumstein et al., 2000). While small numbers of domestic dogs (*Canis familiaris*) and cats inhabit the island, these are typically found in proximity of human settlements and not in the areas inhabited by the wallabies (Blumstein et al., 2000). To our knowledge, none of our animals had ever had any contact with foxes. In contrast, cats are seen occasionally within the Fauna Park.

2.2. Individual test enclosures

Housing and testing conditions have been described previously (Fig. 1 in Griffin et al., 2001). Briefly, each wallaby was held in a small (length 12 m × width 4 m) individual enclosure. The fence was fully screened except for a 1 m × 0.4 m opening which allowed the experimenter to watch and videorecord the animal, and two 1.5 m × 1 m openings (stages), in which visual stimuli were presented. Stimuli were fixed to a cart that ran on inclined rails and could be pulled quietly onto and off the stages by means of a string and pulley system.

2.3. Stimulus models

We used three taxidermically-prepared quadrupedal vertebrate models of approximately equivalent size. A fox was selected to represent an unfamiliar predator and was also the model which was aversively rein-

forced during training. To determine whether experience had a differential effect on responses to a predatory and a non-predatory stimulus, we used a model cat and a model wallaby. Earlier studies have demonstrated that tammars are able to discriminate between these mounts on the basis of morphological cues alone (Blumstein et al., 2000; Griffin et al., 2001). In addition, there is no evidence that wallabies respond differentially to predator odours (Blumstein et al., 2002). Responses to these models were compared with those evoked during a 'blank' control, in which no stimulus was presented, which allowed us to quantify general increases in arousal associated with training.

2.4. Experimental protocol

2.4.1. Acclimatisation

We first habituated each wallaby to foraging while an experimenter was present in the hide by placing a small pile of preferred food (rolled oats) in the centre of the pen. Most animals learnt to forage while being observed within 4–5 days (mean = 4.5 days, range = 3–7 days). Behavioural testing began once the animal had come to feed on two consecutive occasions.

2.4.2. Behavioural testing

Each wallaby first received four pre-training trials during which we measured its initial response to each vertebrate model and the blank control, presented in random order. They then underwent a series of training trials consisting of either paired (experimental group, see below) or explicitly unpaired (control group, see below) presentations of a model fox and a simulated capture, as well as presentations of a cat and a wallaby alone. At the end of the series of training trials, we conducted four post-training trials in which each wallaby was shown the same set of stimuli as in the pre-training trials, but in a different random order. Throughout the study, the stage on which the stimulus appeared was alternated from trial to trial to reduce the likelihood of habituation.

2.4.3. Pre-/post-training trials

We ran two pre-/post-training trials per day, one in the morning and one in the evening. All trials were conducted within 4 h of sunrise or sunset to correspond with the peak periods of diurnal foraging activity (Blumstein et al., 2000). In each trial, a single

stimulus was presented for 60 s on the stage. Stimuli were presented only if the subject was foraging, or engaged in other relaxed behaviour, such as grooming or sitting, and were in a location that afforded them an unobstructed view of the model. These criteria controlled both baseline behaviour and position.

2.4.4. Training trials

Animals were randomly assigned to the paired (experimental) or explicitly unpaired (control) group. The paired group underwent four training trials in which the fox appeared on the stage approximately 3–5 s before a human carrying a net emerged through the hide and began a simulated capture procedure. In this group, the appearance of the fox thus reliably predicted the aversive event. Animals were run four times back and forth in the enclosure along the fence while the net was held just above the ground. We thus mimicked a standard capture procedure, but animals were never caught. The human then exited the yard through the hide and the fox was withdrawn from the stage approximately 2 s later. The whole procedure lasted approximately 60 s. Animals typically resumed relaxed behaviour, such as grooming and foraging, within a few minutes after each training trial.

The unpaired-control group also underwent four simulated capture procedures and was presented with the fox four times, for 60 s each, but these two events were never simultaneous. Instead, they were separated by a minimum of 25 min and a maximum of 12 h. In half of the trials, the animals saw the fox first and in the other half they experienced the simulated capture first. For this group, there was thus no predictive relationship between fox and aversive event.

In addition to paired or unpaired presentations of fox and simulated capture, both experimental and control groups of wallabies experienced four 60 s presentations of a cat alone and four 60 s presentations of a conspecific wallaby alone.

We conducted three (experimental group: fox + simulated capture, cat, wallaby) or four training trials (control group: fox, simulated capture, cat, wallaby) per day. Trials were conducted in a random order and separated by a minimum of 25 min and a maximum of 12 h. During non-reinforced presentations, the experimenter remained out of the sight of the subject. These trials were conducted independently of whether the wallaby was foraging or not.

The total experience of wallabies in the paired and the unpaired groups was identical in all respects (i.e. total exposure to all stimuli and simulated capture attempts was matched), except for the fox–human contingency, which was experienced by the paired group only. Planned comparisons between paired and unpaired groups using response differences between pre- and post-training trials allowed us to detect changes specifically attributable to associative learning (Shettleworth, 1998) and to separate such effects from those that might be a consequence of other factors, such as confinement in the test yards and repeated exposure to the models. We expected the responses evoked by the fox to increase significantly in the experimental group, relative to the control group, because this stimulus reliably predicted the simulated capture event.

2.5. Data analysis

Data were collected during pre- and post-training trials only and analysed in the same way as in our earlier fox-training study (Griffin et al., 2001). We videorecorded wallabies for 1 min immediately prior to stimulus presentation (baseline), 1 min during the stimulus presentation, and 5 min after the stimulus had disappeared from the stage. The 3–5 s interval during which the stimulus was moving along the track, but not yet visible to the wallaby, was excluded from analyses.

We scored behaviour from videorecordings to 0.1 s resolution using The Observer 3.0 (Noldus Information Technologies, 1995). Behaviours were scored as either vigilance (bipedal stand), relaxed (forage, sit with tail between legs, groom) or locomotion (hop, pentapedal walk). Quadrupedal crouching can either be a relaxed behaviour (e.g. sunbasking), or an alert behaviour (e.g. brief crouching stance between two hops), so it was not quantified.

To assess responses to each stimulus, we measured changes in behaviour from pre-stimulus baseline. Reductions in relaxed behaviour reflect all transitions to an alarmed state, which is typically characterised by alternating bouts of vigilance and locomotion. Both of these behaviours are adaptive if faced with a predator. Relaxed behaviour thus provides an aggregate measure that is a particularly sensitive assay for assessing overall antipredator response.

To measure changes in behaviour from pre-stimulus baseline, the percentage of time spent in vigilance, locomotion and relaxed behaviour was determined for the 60 s baseline and for 24 successive 15 s time intervals after stimulus onset. We then calculated difference scores for each 15 s interval, relative to the pre-stimulus baseline. To quantify the effects of training, we calculated the change in percentage of time spent in each behaviour between pre- and post-training trials (*pre-/post-response difference*) for each stimulus, group and 15 s time interval. We then compared the paired group's mean pre-/post-response difference to that of the unpaired group using a two-way repeated-measures ANOVA with factors for group (paired/unpaired) and time (successive 15 s intervals).

All statistical analyses were carried out on untransformed data using Statview 5.1 (SAS Institute, 1998) and Superanova 1.1 (Abacus Concepts Inc, 1991). Since sequential measures of behaviour are typically more highly correlated than more temporally distant measures, we used Huyn–Feldt adjusted *P* values for all analyses with a time factor (Huyn and Feldt, 1976). Comparisons involving responses to the fox in the paired-experimental versus the unpaired-control group, for which we had a priori predictions, were one-tailed; all others were two-tailed. We used an alpha level of 0.05 throughout.

3. Results

ANOVAs comparing the mean pre-/post-response difference of the paired-experimental group with that of the unpaired-control group for each stimulus revealed no significant main effects for the time factor (i.e. 15 s intervals), nor any significant group × time interactions. For this reason, we collapsed the mean pre-/post-response difference over time (Figs. 1 and 2) and report only the main effects for the group (paired versus unpaired) factor (Table 1).

After training, wallabies in the paired-experimental group suppressed relaxed behaviour (Fig. 1) and increased vigilance (Fig. 2) significantly more in response to the fox model than unpaired-controls (Table 1). Despite repeated non-reinforced presentations, the cat also significantly increased vigilance and reduced relaxed behaviour in the experimental group, relative to controls (Figs. 1 and 2; Table 1).

Table 1
Comparison of experimental and control groups

	Stimulus	Group main effect	
		$F_{(1,14)}$	P
Relaxed behaviour	Fox	12.413	0.002[a]
	Cat	5.806	0.030
	Wallaby	3.182	0.096
	Blank	0.188	0.671
Vigilance	Fox	9.247	0.004[a]
	Cat	5.317	0.037
	Wallaby	1.947	0.185
	Blank	0.069	0.796
Locomotion	Fox	0.521	0.241[a]
	Cat	1.934	0.186
	Wallaby	0.705	0.415
	Blank	1.476	0.245

Results for the group factor from two-way repeated-measures ANOVAs (group × time) comparing the mean pre-/post-response difference for each behaviour. There were no significant main effects of time or group × time interactions for any stimulus (see text for details).

[a] One-tailed comparisons, all others are two-tailed.

In contrast, there were no significant differences in responses to the conspecific wallaby stimulus, even though this model had also been repeatedly presented alone during training, in the same way as the cat (Figs. 1 and 2; Table 1). There were also no differences between the two groups during the blank trials, demonstrating that training did not cause a general increase in arousal (Figs. 1 and 2; Table 1). This pattern of results demonstrates that wallabies learnt quite specifically that the appearance of a predator model predicted the onset of the capture attempt, and that the appearance of a wallaby model did not.

There were no significant differences in locomotion between the two groups in response to any stimulus (Table 1).

4. Discussion

After undergoing training in which a fox model was paired with simulated capture, wallabies became significantly less relaxed and more vigilant in response to the predator, relative to a control group which was matched for total experience with all stimuli, but which had not experienced a predictive relationship between the fox and an aversive event (Table 1). A cat evoked
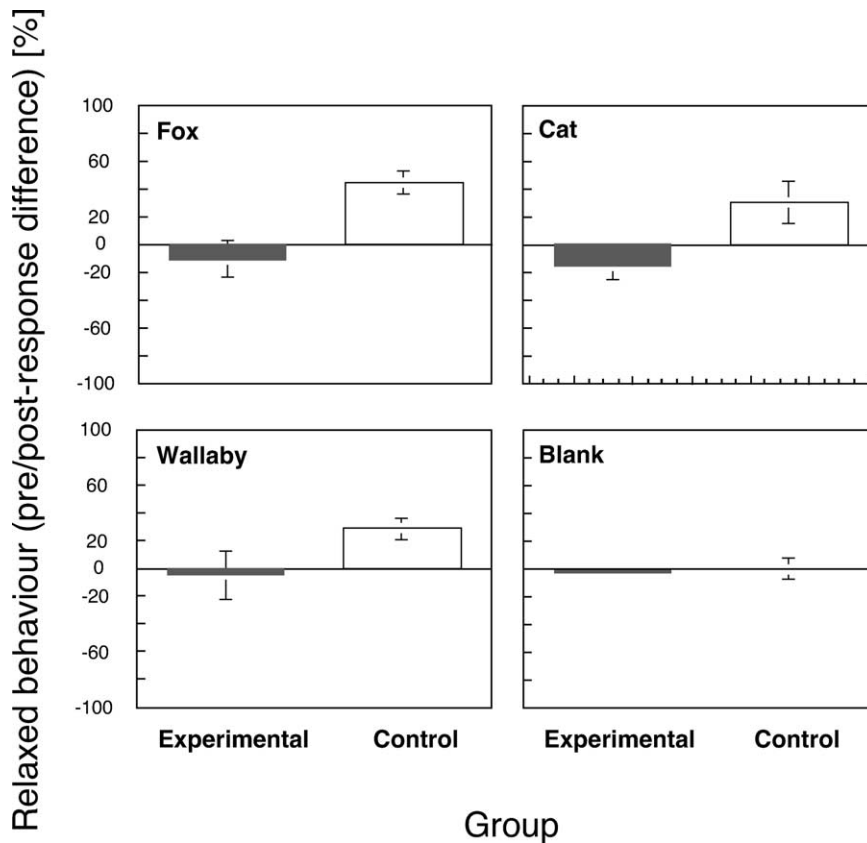
Fig. 1. Changes in relaxed behaviour after training for the paired-experimental ($N = 8$) and unpaired-control groups ($N = 8$). The mean pre-/post-response difference ($\pm$S.E.) is plotted for both groups. Note that enhanced response as a consequence of training will be reflected in a reduced proportion of relaxed behaviour (see text for details). In some cases, standard errors are smaller than histogram bars.

a similar response to that elicited by the fox, despite repeated non-reinforced presentations of this predator model during training (Table 1). In contrast, there were no significant differences between the two groups in response to a conspecific wallaby (Table 1), although the experience of subjects with this model was matched to that with the cat.

In an earlier study, we conducted identical pairings of fox and simulated capture, but did not present a conspecific model during training (Griffin et al., 2001). This procedure increased the vigilance response to the fox, and also to a model wallaby, although briefly (Griffin et al., 2001). There were no changes in behaviour toward the conspecific stimulus in the present study, suggesting that differential reinforcement of the predator and the conspecific model

enhanced the specificity of the acquired antipredator response.

There is a clear contrast with post-training responses to the model cat. Despite repeated non-reinforced presentations of this stimulus, the response of the wallabies resembled that to the aversively-reinforced fox. The difference between the experimental and control groups' responses to the cat necessarily reflects the effect of the contingency between fox and simulated capture. Wallabies thus generalised their acquired response from the predator that was aversively reinforced during training to one that was not, and this effect was not eliminated by repeated non-reinforced presentations. This finding is consistent with those of our earlier study in which we also found generalisation from the fox, which was aversively reinforced
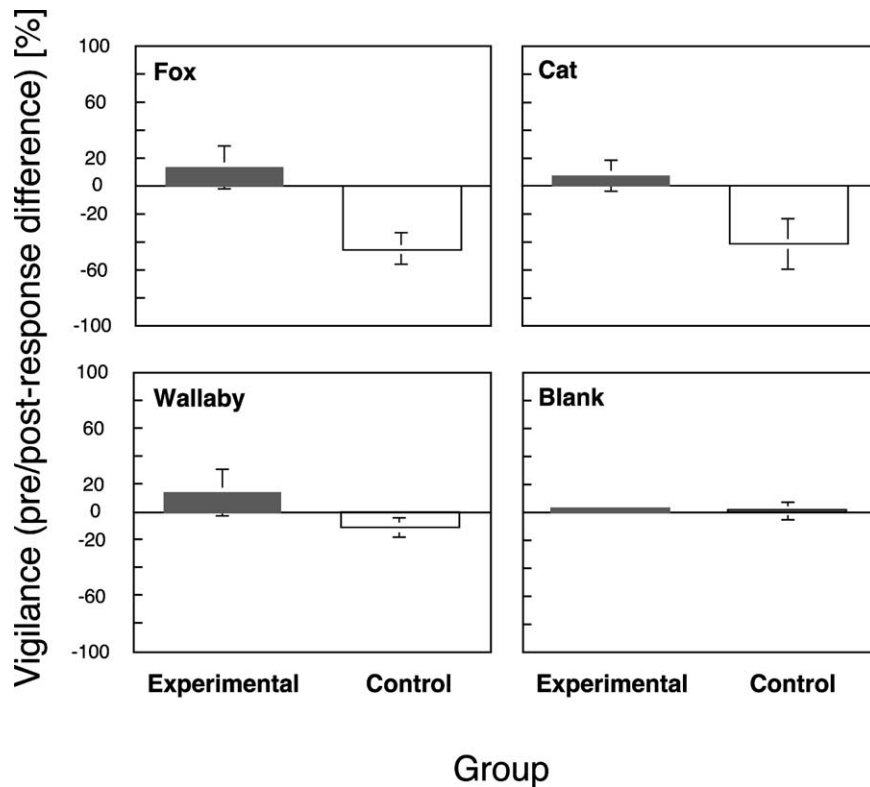
Fig. 2. Changes in vigilance after training for the paired-experimental and unpaired-control groups. The mean pre-/post-response difference (±S.E.) is plotted for both groups. In some cases, standard errors are smaller than histogram bars.

during training, to a cat, which was not (Griffin et al., 2001). The present results suggest that the tendency of wallabies to generalise the effects of individual experience is sufficiently robust to resist the effects of habituation.

It has been predicted that differential reinforcement of dangerous and harmless stimuli by conspecific alarm behaviour should enhance the specificity of antipredator responses (Seyfarth and Cheney, 1986; Curio, 1993). Our findings provide support for this idea, with the difference that enhanced antipredator responses resulted from individual learning, rather than from social learning. Our results also reveal that differential reinforcement did not inculcate differential responses to two predators, presumably because the effect of generalisation overrode that of habituation. We conclude that the relative strength of these effects is influenced by stimulus properties, which is consistent with earlier findings suggesting that

tammars perceive predators as qualitatively distinct (Blumstein et al., 2000; Griffin et al., 2002).

quarie University Animal Ethics Committee (protocol #98038).

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