



Learning specificity in acquired predator recognition

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Predator recognition is often dependent upon experience. This behavioural plasticity can potentially be exploited to enhance the antipredator behaviour of captive-bred animals, but it is first necessary to understand the specificity of learning. We enhanced the responses of tammar wallabies, *Macropus eugenii*, to a model fox, *Vulpes vulpes*, by presenting this novel predator in conjunction with a human simulating a capture procedure. A control group had identical total exposure to fox and human, but with no such predictive relationship between these two events. Animals that experienced paired presentations of fox and human behaved more cautiously towards the fox after training than controls. To assess whether this learnt response was specific to the fox, we presented the animals with an array of visual stimuli both before and after training. The tammars generalized their acquired response from the predator with which they were trained to a predator with which they were not trained (cat, *Felis catus*), but not to a nonpredator (goat, *Capra hircus*). Tammars also exhibited a transient increase in response to a model wallaby after training. We suggest that this effect is more likely to reflect social behaviour than generalization of the learnt response from predator to conspecific. Two additional controls revealed that changes in behaviour after training were not attributable to the presentation device and were not caused by a general decrease in response threshold associated with training. Our results suggest that tammar wallabies perceive predators as a natural category.

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Although it may seem counterintuitive for antipredator skills to be dependent upon experience, the ability to learn about previously unfamiliar predators has been demonstrated in a wide range of taxa including fish, birds and primates (reviewed by Griffin et al. 2000). Predator-learning studies have been undertaken within both theoretical and applied frameworks. Determining whether animals can learn about novel predators and, if so, investigating how they learn, provides insights into learning mechanisms. The results of experimental studies strongly suggest that certain types of stimuli may be particularly effective at triggering learning about predators. For instance, conspecific alarm signals or fear responses may facilitate learning in other individuals present (Curio 1988; Mineka & Cook 1988; Maloney & McLean 1995). In addition, there is evidence that some stimuli are more readily associated with fear than others. Acquired responses are often less strong if learning is attempted with biologically irrelevant stimuli (Curio 1988; Mineka & Cook 1988; Magurran 1989).

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The question of how readily animals learn about predators is also important from an applied perspective. Reintroduced and translocated individuals are particularly vulnerable to predation after release, which has reduced the success of conservation programmes (MacMillan 1990; Beck et al. 1994; Wolf et al. 1996). Prerelease antipredator training has consequently been used to try to improve antipredator skills (Ellis et al. 1977; Miller et al. 1990; Maloney & McLean 1995; McLean et al. 1996, 1999, 2000; McLean 1997; Richards 1998). Responses to predators have been successfully enhanced with a range of conditioning techniques in which individuals learn to associate the target predator with an unpleasant experience (reviewed by Griffin et al. 2000).

While both theoretical (Dill 1974; Herzog & Hopf 1984; Curio 1988; Mineka & Cook 1988; Magurran 1989; Chivers & Smith 1994; Berger et al. 2001) and applied (Ellis et al. 1977; Miller et al. 1990; Maloney & McLean 1995) studies have established that many species are able to learn about novel predators, the issue of learning specificity has been largely neglected. What exactly is learnt during training, and how specific to the target predator is the enhanced response?

This question can be addressed in several ways. First, to verify that post-training responses are not solely a consequence of a general increase in wariness, control subjects that are not given the opportunity to associate the target predator with an unpleasant event need to be incorporated into experimental designs. With a few exceptions (Maloney & McLean 1995; McLean et al. 1999), applied studies have not measured post-training responses of control animals (Ellis et al. 1977; Miller et al. 1990; McLean et al. 1996, 2000). Second, to assess the specificity of trained responses it is necessary to quantify the effects of presenting control objects. Some studies have measured post-training responses to biologically irrelevant control objects (e.g. wood blocks, plastic bottles; Mineka & Cook 1988; McLean et al. 1999), but few have used biologically meaningful stimuli (although see Kramer & von St Paul 1951). It is consequently not clear whether learnt responses will be generalized from the target predator to a similar natural stimulus, such as a nonpredator.

Studies of learning specificity are of theoretical interest because they provide insights into how animals categorize stimuli. While there are abundant empirical data on generalization of learnt responses, these studies have been carried out on a limited number of species and focus almost exclusively on simple artificial stimuli, such as lights and simple sounds (reviewed by Shettleworth 1998). Although recognition of natural objects has been explored in several systems (e.g. Curio 1993; Jedrzewski et al. 1993; Evans & Marler 1995), much less is known about how animals categorize complex stimuli about which they have learnt (although see Kramer & von St Paul 1951; Herrnstein et al. 1976; Herrnstein 1979; Real et al. 1984).

Learning specificity also has important applied implications, as it is likely to influence substantially the success of predator training in conservation programmes. After release, trained responses will extinguish quickly if they are continuously evoked by nonpredatory stimuli because they will not be reinforced (reviewed by Shettleworth 1998). Conversely, if trained responses are specific to predators, then they will be expressed more rarely and strongly reinforced. If potential prey survive the encounter, then such responses are more likely to be maintained permanently. In summary, some degree of response generalization from trained to nontrained predators is likely to be advantageous, while greater response generalization to include other stimuli is likely to be detrimental. For this reason, we have suggested that assessment of learning specificity should be an essential aspect of antipredator training (Griffin et al. 2000).

On mainland Australia, introduced foxes, *Vulpes vulpes*, and feral cats, *Felis catus*, pose a major threat to small and medium-sized marsupials; ca. 20% of species and subspecies are consequently either extinct or severely threatened (Maxwell et al. 1996). Some species survive only on offshore islands that have been historically isolated from mammalian predators. Reintroductions and translocations are common conservation management procedures for macropods and other marsupials (Serena 1995; Maxwell et al. 1996) and it is necessary to develop

prerelease procedures that maximize postrelease survival rates.

We used tammar wallabies as a model species of marsupial to investigate the specificity of learning in acquired predator recognition. We selected tammars for several reasons. First, they adjust well and reproduce readily in captivity, which makes them particularly suited to experimental studies. Second, individuals from an isolated island population have retained sophisticated antipredator behaviour and predator recognition, even though they have had no contact with mammalian predators for the past 9500 years (Blumstein et al. 1999, 2000). This is important because it should be easier to train animals that already have some pre-existing components of antipredator behaviour (Griffin et al. 2000). Third, we have developed an optimal antipredator training method for this species during an extensive pilot study (A. S. Griffin, unpublished data), which involved testing the effects of a range of variables (e.g. number of training trials, spatial relationship between stimuli) known to influence the likelihood of learning in animals (Shettleworth 1998). Fourth, few studies have investigated mechanisms of learning in marsupials (McLean et al. 1996; Wynne & McLean 1999), in contrast to the large literature on learning in eutherian mammals. Finally, there is a specific interest in developing antipredator training techniques for tammar wallabies because there are plans to reintroduce island individuals to mainland Australia where they will face evolutionarily novel predators (foxes and cats).

Our goal was to increase the antipredator responses of tammars to foxes by pairing the presentation of a taxidermically prepared model fox with an aversive stimulus (Training). Social alarm signals are effective for triggering learning about predators in some species (Curio et al. 1978), but pilot trials showed that pairing conspecific alarm thumps with a predator model did not produce learning in tammars (A. S. Griffin, unpublished data). We consequently selected a human carrying a net as an aversive stimulus. Approaching humans consistently elicit alarm responses in captive marsupials, probably because animals associate them with being caught, bagged and handled, which are common management procedures. Simulated capture was not used to emulate the experience of attack by a real predator, but rather to provide a standard stimulus that was sufficient to elicit a high-level alarm response. This approach allowed a controlled assessment of the properties of learning about predators without the ethical concerns that would be raised by using more potent aversive stimuli (Griffin et al. 2000).

To determine whether the tammars' acquired responses were specific to the fox, we also quantified responses to an array of visual models, both before and after training (Pretraining and Post-training trials). Our design also incorporated an 'unpaired'-control group in which animals had identical total exposure to the fox and human, but with no predictive relationship between these two stimuli. Comparisons between the responses of the paired and unpaired groups allowed us to isolate changes attributable specifically to learning (Shettleworth 1998).

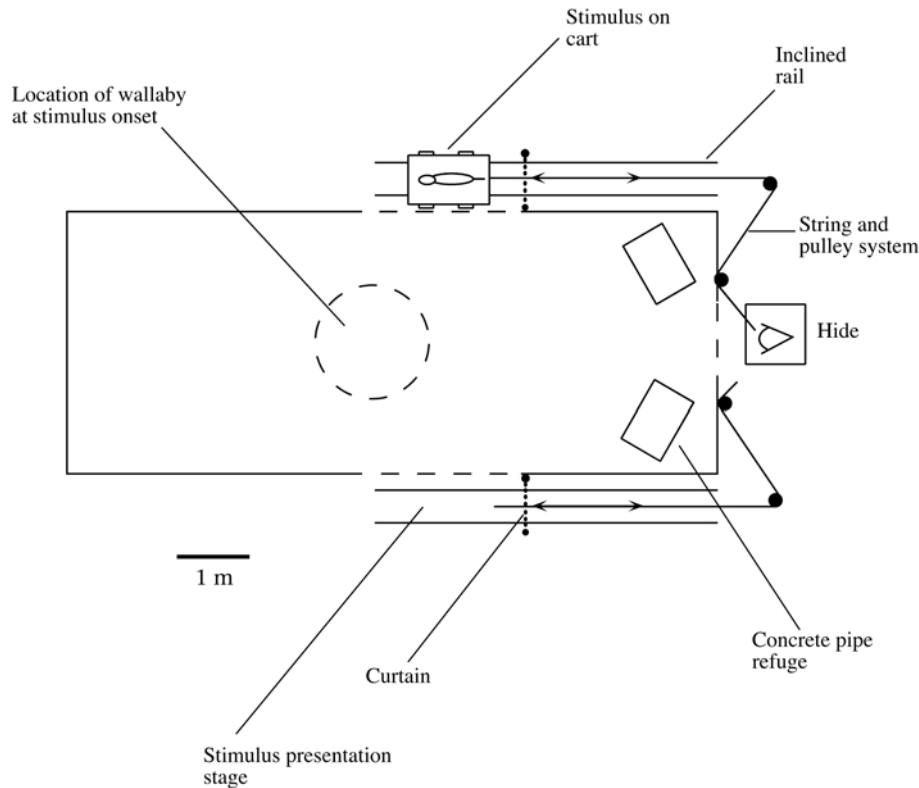


Figure 1. Plan view of an individual test yard. Concrete pipes provided the wallabies with shelter. Visual stimuli were presented only if the subject was foraging or engaged in other relaxed behaviour in the centre of the pen (see text for details).

METHODS

Subjects and Husbandry

We used 16 adult tamar wallabies (11 females and five males). All animals were bred at the Macquarie University Fauna Park and had been caught and handled regularly prior to the experiments. They were obtained from large social groups and returned to these after they had completed the experiments. While in social groups, the wallabies were held in enclosures of 30×30 m. Males were in all-male groups to avoid aggression caused by the presence of females, whereas females were in breeding colonies containing ca. 15 individuals and one male. The wallabies had access to food (kangaroo pellets) and water ad libitum. The yards had natural vegetation and were provided with concrete pipes for additional shelter. To our knowledge, none of these animals had ever had any contact with foxes. In contrast, cats are seen occasionally within the Fauna Park. All husbandry and experimental procedures were approved by the Macquarie University Animal Ethics Committee.

Individual Test Yards

During experiments, each animal was held in an individual test yard. These were wire-fenced enclosures (length $12 \text{ m} \times$ width 4 m ; Fig. 1). Two large concrete pipes (length 1 m , diameter 0.70 m) were provided for shelter. The fence was screened with a 2-m -high strip of opaque black plastic 'weedmat' to isolate the animal

visually from its surroundings. An opening of $1 \times 0.4 \text{ m}$ in the plastic on one of the short sides of the enclosure allowed the observer to watch and videorecord the animal from a hide abutting the fence. Additional openings of $1.5 \times 1 \text{ m}$ were located in the middle of each of the long sides of the enclosure, and behind these were stages on which visual stimuli were presented (Fig. 1). The fence in front of each stage was painted black to reduce light reflection and optimize visibility through the wire. Stimuli were fixed to a cart that ran on inclined rails and could be pulled quietly on to and off the stages by means of a string and pulley system, which was operated by the experimenter from the hide (Fig. 1). Curtains hanging perpendicular to the rails on each side screened stimuli from the wallaby before and after presentations.

Experimental Protocol

Acclimatization

After transfer from large group enclosures to individual test yards, it was necessary to habituate the animals to foraging while an experimenter was in the hide. Each morning and evening, the experimenter entered the enclosure through the hide window and placed a small pile of preferred food (rolled oats) in the centre of the pen. She then watched the subject for $5\text{--}10$ min from the hide. The majority of animals learnt to forage while being observed within $4\text{--}5$ days (mean 4.8 days, range $3\text{--}8$ days). Behavioural testing began once the animal had come to feed on two consecutive occasions.

Experiments

Each wallaby first received six pretraining trials during which we quantified its initial response to an array of stimuli, presented in random order. We then conducted four training trials in which a model fox was either paired (experimental group, see below) or explicitly unpaired (control group, see below) with an aversive event, namely a simulated capture procedure. Extensive pilot work with other subjects had shown that tammars tended to habituate to the simulated capture procedure if training was extended beyond four trials, presumably because the animals were never caught, but that fewer training trials were not sufficient for learning to occur (A. S. Griffin, unpublished data). After training, the animals underwent six post-training trials in which they were shown the same set of stimuli as in the pretraining 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.

Pre- and post-training trials

We elected to use stimulus models for reasons of experimental control. Models are an effective technique for studying predator recognition and its acquisition (e.g. Curio 1988) and work in our laboratory has shown that our taxidermic mounts elicit biologically meaningful responses in tammars (Blumstein et al. 2000). Since models were presented in a standard way, differences in response after training could be attributed to variation in stimulus morphology, rather than to dynamic cues, such as gait. To the extent that having only a subset of cues made the stimuli more difficult for the wallabies to discriminate, our test is likely to be conservative (i.e. to underestimate the potential specificity of acquired responses).

Models were selected to tease apart the effects of training, familiarity and predatory threat. A fox was an unfamiliar predator and also the model with which the animals were trained. To determine whether the effects of fox training generalized to another species of predator, we used a model cat. A model juvenile goat enabled us to compare responses to a predator and a nonpredator without the confound of novelty because both the fox and the goat were initially unfamiliar. Finally, we showed the animals a model wallaby to determine the effects of presenting a vertebrate stimulus that was neither novel nor a predator. All four vertebrates were of similar size and mounted in a quadrupedal stance. Previous data collected in our laboratory suggested strongly that olfactory cues associated with the taxidermic mounts (which had no smell to us) would not explain variation in the animals' responses (Blumstein et al. 2000). We compared responses to these models with those evoked by two control stimuli. The cart was presented alone to measure the proportion of response attributable to the presentation device and its associated movement. Finally, we conducted 'blank' trials, in which no stimulus was presented, to quantify general changes in behaviour associated with training.

Training trials

We randomly assigned animals to the paired (experimental) or explicitly unpaired (control) group. The paired group underwent training trials in which the fox appeared on the stage ca. 3–5 s before a human carrying a net emerged through the hide and began a simulated capture procedure (Fig. 2). In this group, the appearance of the fox thus reliably predicted the onset of the capture 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 the animals were never caught. The human then exited the yard through the hide and the fox was withdrawn from the stage ca. 2 s later. The whole procedure lasted ca. 60 s. Animals typically resumed relaxed behaviour, such as grooming and foraging, within a few minutes of 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 (Fig. 2). Instead, they were separated by a minimum of 25 min and a maximum of 90 min. In half 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 an aversive event.

Note that experience of wallabies in the paired and the unpaired groups was identical in all respects (i.e. total exposure to the predator stimuli and simulated capture attempts were matched), except for the fox–human contingency, which was experienced by the paired group only (Fig. 2). Planned comparisons of post-training responses to the fox thus allowed us to detect changes specifically attributable to 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 predator models.

Test procedure

All data were collected within 4 h of sunrise and 4 h of sunset. These are ideal times to run trials because tammars, while primarily nocturnal, forage in the mornings and in the late afternoon (Blumstein et al. 1999). We ran two pre-/post-training trials per day, one in the morning and one in the evening, but training trials were conducted in the evening only. The observer was present in the hide throughout all pre- and post-training trials, so presence of a human was not confounded with treatment. A single stimulus was presented for 60 s on the stage during each trial. Stimuli were presented only if the subject was foraging or engaged in some other relaxed behaviour such as grooming or sitting. This approach controlled both baseline behaviour and the location of the animals when they first saw the visual stimuli. If the wallaby had not come to feed within 2 h, the trial was postponed until the next scheduled test time. However, trials were not postponed for more than 2 days to control for maximum time interval between successive stimulus presentations. Upon completion of the post-training trials, all subjects were returned to their social groups; we detected no signs of stress after their return.

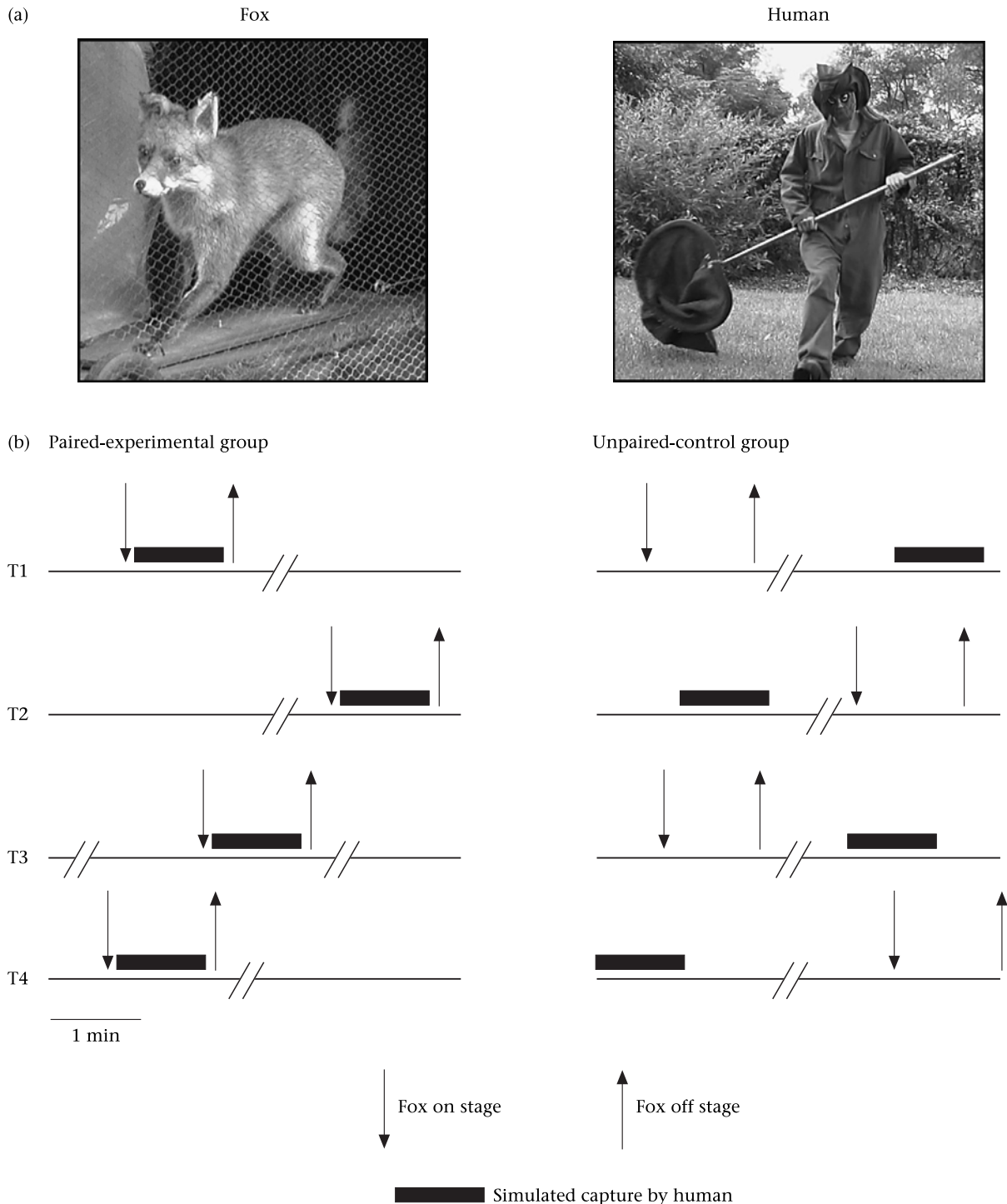


Figure 2. Training technique. (a) Stimuli used during training. The aversive human wore overalls, a hat and a mask to ensure that she was morphologically distinct from the observer in the hide. (b) Schematic representation of training procedure for the paired-experimental and unpaired-control groups. T1–T4 indicate four successive training trials, which were conducted at a different time each evening. In the paired group, the fox appeared on the stage 3–5 s before the human with the net entered the yard. In contrast, wallabies in the unpaired-control group saw the fox and human with an intervening interval of 25–90 min and in no consistent order. The two groups thus had identical overall experience of fox presentations and simulated captures, but there was a predictive relationship between these two events for the paired group only.

Data Analysis

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.

Behaviours were grouped into three categories: vigilance (bipedal stand), relaxed (forage, sit with tail between legs, groom) and locomotion (hop, pentapedal walk). Quadrupedal crouching can be either a relaxed behaviour (e.g. sunbasking), or an alert behaviour (e.g. brief crouching stance between two hops); it was consequently not included in analyses. We scored test videorecordings, using continuous sampling to 0.1-s resolution, with The Observer 3.0 (Noldus Information Technologies 1995).

To quantify overall antipredator response to each stimulus, we measured changes in behavioural time budgets over time. In our experimental setting, an alarmed state was typically characterized by bouts of vigilance, interspersed with brief bouts of locomotion. Reductions in relaxed behaviour reflect the sum of all transitions to an alarmed state because vigilance, locomotion and relaxed behaviour are all mutually exclusive. Relaxed behaviour hence provides an aggregate measure which is likely to be the most sensitive assay for quantifying overall antipredator response. To characterize further the nature of the animals' alarm responses, we also examined each component (vigilance and locomotion) separately. Initial statistical analyses were conducted with relaxed behaviour as a dependent variable. Vigilance and locomotion were analysed statistically only for those stimuli that had elicited significant changes in relaxed behaviour.

The percentage of time allocated to relaxed behaviour, vigilance and locomotion was determined for the 60-s baseline and for 24 successive 15-s intervals after stimulus onset. We then calculated difference scores for each 15-s interval, relative to the prestimulus baseline. To quantify the effects of training, we calculated the change in the percentage of time spent in each behaviour between pre- and post-training trials (pre-/postresponse difference) for each stimulus, group and 15-s time interval. We then compared the paired group's mean pre-/postresponse difference to that of the unpaired group with a two-way repeated measures ANOVA with factors for group (paired and unpaired) and time (successive 15-s intervals). These analyses were conducted separately for each of the six pre-/post-test stimuli.

We expected changes in locomotion to occur principally at stimulus onset and to wane quickly thereafter. To detect such effects, we focused upon changes in locomotion in the first 15-s time interval by using Mann–Whitney *U* tests to compare the paired group's mean pre-/postresponse difference with that of the unpaired group. These tests were conducted only for stimuli that produced significant changes in relaxed behaviour to limit the total number of comparisons. We refer to locomotion during the entire 6-min trial as sustained

locomotion and to locomotion during the first 15-s time interval as immediate locomotion.

To identify further the specificity of training effects, we also carried out planned comparisons between pairs of stimuli using three-way (group, stimulus, time) repeated measures ANOVAs. First, we compared the level of relaxed behaviour during and after fox presentations with that in the cat trials. This analysis assessed the degree to which fear responses generalized from the predator used during training to one that had not predicted an aversive event. Second, we made an analogous comparison between responses to the fox and to the goat to determine whether tammar generalized their acquired fear response to a novel nonpredator. Third, we compared responses to the fox with those to the wallaby to determine whether effects of predator presentations differed from those of a familiar nonpredator. Finally, we compared responses to the fox with those to each of the control stimuli (cart, blank) to assess whether changes in behaviour associated with the trained predator could have occurred as a consequence of the presentation device, or of a general decrease in response threshold associated with training.

All statistical analyses were carried out on untransformed data using Statview 5.1 (SAS Institute 1998) and Superanova 1.1 (Abacus Concepts 1991). Since sequential measures of behaviour are typically more highly correlated than more temporally distant measures, we report Huyn–Feldt adjusted *P* values for all analyses involving a time factor (Huyn & Feldt 1975). 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 of 0.05 throughout. For each between-stimulus planned comparison, we also calculated an effect size using Cohen's *d* (Cohen 1988).

RESULTS

After training, presentation of the fox caused a sustained reduction in relaxed behaviour, relative to the unpaired control (Fig. 3). The cat evoked a similar response, while the effects of wallaby presentation were relatively transient (Fig. 3).

Statistical analyses reveal that animals in the paired group, in which the fox had predicted the onset of a capture procedure, suppressed relaxed behaviour significantly more in response to this model than the unpaired controls, which had not experienced this contingency (Table 1). Although the cat and the wallaby had never been paired with an aversive event, these stimuli also significantly suppressed relaxed behaviour in the paired group, relative to the unpaired group (Table 1). In contrast, there were no significant differences between the paired and unpaired groups for the goat, or for the cart and blank controls (Table 1). Comparisons between paired and unpaired groups thus reveal that training to the fox led to significant changes in relaxed behaviour to another predator and to a conspecific, but not to a nonpredator. Pairwise comparisons of relaxed behaviour elicited by selected stimuli reveal a significant Group × Stimulus interaction for the fox versus blank

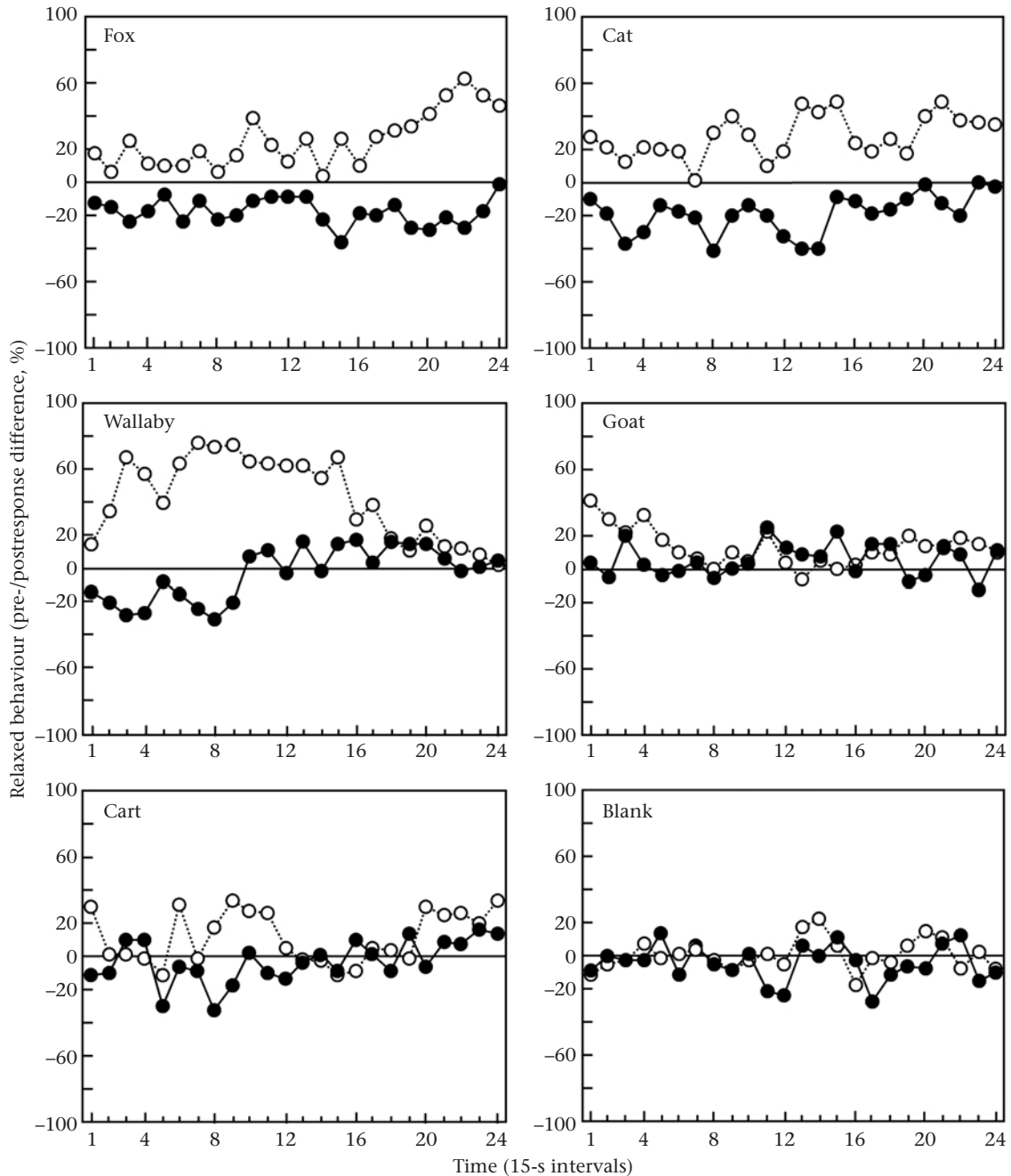


Figure 3. Changes in relaxed behaviour after training for the paired-experimental (●, *N*=8) and unpaired-control (○, *N*=8) groups. The mean pre-/postresponse difference is plotted for 24 15-s time intervals from stimulus onset, 1 min during stimulus presentation and 5 min after the stimulus had disappeared from the stage. Note that enhanced responses to the predator will be reflected in a reduced proportion of relaxed behaviour (see text for details).

control (Table 2). This indicates that the trained response to the fox does not simply reflect a general increase in wariness associated with training.

Changes in relaxed behaviour as a consequence of training were largely reflected in main effects for groups (paired versus unpaired) in the within-stimulus comparisons (Table 1). However, there was also a significant Group × Time interaction for the wallaby (Table 1), but not for any of the other stimuli. There was also a significant Group × Stimulus × Time interaction when levels of

relaxed behaviour during and after wallaby presentations were compared with those in fox trials (Table 2). Taken together, these results reveal differential change in behaviour over time and suggest that the effect of training was to inculcate a sustained reduction in relaxed behaviour to the fox and cat, but a relatively brief change to the conspecific stimulus.

When the animals suppressed relaxed behaviour, they switched to alert behaviour that combined bouts of vigilance (Fig. 4) and locomotion (Fig. 5). For those stimuli

Table 1. Within-stimulus comparisons: results of a two-way repeated measures ANOVA (Group×Time) comparing the pre-/postresponse difference in three behaviours of the paired-experimental group with that of the unpaired-control group for each stimulus

Stimulus	ANOVA factor			
	Group main effect		Group×Time interaction	
	$F_{1,23}$	P	$F_{1,23}$	P^*
Relaxed behaviour				
Fox	7.867	0.007†	1.250	0.274
Cat	7.286	0.017	0.670	0.728
Wallaby	9.149	0.009	3.458	<0.001
Blank	0.069	0.796	0.625	0.874
Cart	0.890	0.361	1.032	0.423
Goat	0.235	0.635	0.832	0.622
Vigilance				
Fox	3.705	0.035†	0.686	0.679
Cat	11.779	0.004	1.234	0.270
Wallaby	6.484	0.023	2.761	0.003
Sustained locomotion				
Fox	2.386	0.072†	1.169	0.319
Cat	0.496	0.493	1.286	0.256
Wallaby	4.397	0.055	2.397	0.008

Sustained locomotion refers to elevated levels throughout the 6-min trial. For immediate locomotion directly after stimulus presentation, statistical results are indicated in the text.

*Huyn-Feld corrected (see text).

†One-tailed comparisons, all others are two tailed.

Table 2. Between-stimulus comparisons: planned pairwise comparisons between selected pairs of stimuli using pre-/postresponse differences in relaxed behaviour for paired and unpaired groups in a three-way repeated measures ANOVA (Group×Stimulus×Time)

Pair of stimuli		Group×Stimulus			Group×Stimulus×Time interaction	
		Interaction		Effect size d	$F_{1,23}$	P^*
		$F_{1,23}$	P			
Fox	Cat	0.028	0.869	0.09	0.973	0.456
Fox	Goat	3.246	0.093	0.91	0.990	0.453
Fox	Cart	1.520	0.238	0.62	1.206	0.274
Fox	Blank	5.118	0.040	1.13	0.856	0.609
Fox	Wallaby	0.019	0.892	0.07	3.778	<0.001

*Huyn-Feld corrected (see text).

that had produced a significant reduction in relaxed behaviour after training (fox, cat and wallaby), we analysed vigilance and locomotion separately to determine the relative contribution of each of these behaviours to the overall increase in alertness. These analyses reveal that, after training, presentation of the fox produced a transient increase in locomotion that was significantly greater in the paired group than in the unpaired-control group (immediate locomotion: $U=10.0$, $N_1=N_2=8$, $P=0.01$). The difference in locomotion between the two groups waned after the first 15-s time interval (sustained locomotion: Fig. 5, Table 1). Comparison of paired and unpaired groups also reveals a significant difference in sustained vigilance to the fox (Fig. 4, Table 1). The cat also elicited a sustained increase in vigilance (Fig. 4, Table 1), but there was no difference between the two

groups in either immediate ($U=23.0$, $N_1=N_2=8$, $P=0.341$) or sustained (Fig. 5, Table 1) locomotion. Finally, the wallaby evoked a significant, but transient, increase in vigilance in the paired group relative to the unpaired controls (Fig. 4, Table 1), but this was not accompanied by differences in either immediate ($U=31.5$, $N_1=N_2=8$, $P=0.957$) or sustained (Fig. 5, Table 1) locomotion. Alarm thumps were a rare behaviour in both pre- and post-training trials and were too infrequent to be analysed statistically.

DISCUSSION

After training, wallabies became more cautious towards the fox and suppressed relaxed behaviour such as foraging, grooming and sitting both during presentations and

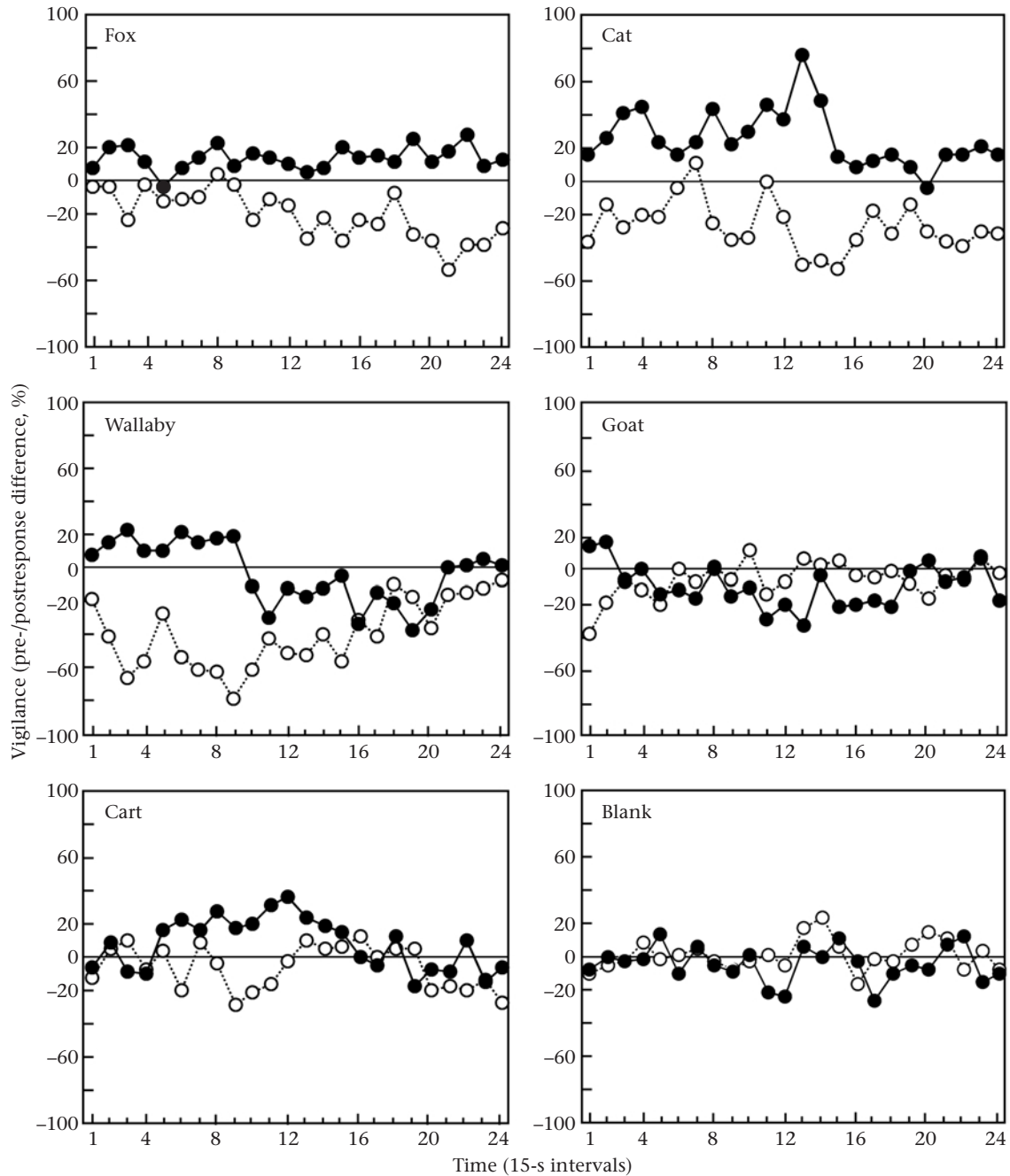


Figure 4. Changes in vigilance after training for the paired-experimental (●, N=8) and unpaired-control (○, N=8) groups. Mean differences have been calculated in the same way as for Fig. 3.

for some minutes afterwards (Fig. 3). Responses to this model were characterized by a brief increase in locomotion immediately after it had appeared, followed by a prolonged increase in vigilance (Fig. 4). Although the cat had never been paired with the aversive stimulus, the same reduction in relaxed behaviour also occurred in response to this model (Fig. 3), together with a prolonged increase in vigilance (Fig. 4). In contrast, animals did not alter their responses to the model goat or the presentation cart after training (Figs 3, 4, 5). Analyses of behaviour during the blank trials reveal that differences between the

paired and the unpaired group were not a consequence of a general increase in vigilance and activity due to training. This pattern of results demonstrates that wallabies learned quite specifically that a model predator predicted the appearance of a human and the onset of the simulated capture procedure (Table 1). Previous studies using simultaneous presentations of a human and a model predator have not been as successful in inculcating a fear response in a marsupial (McLean et al. 2000), probably because the human overshadowed (Shettleworth 1998) the training stimulus. Comparisons with our study thus

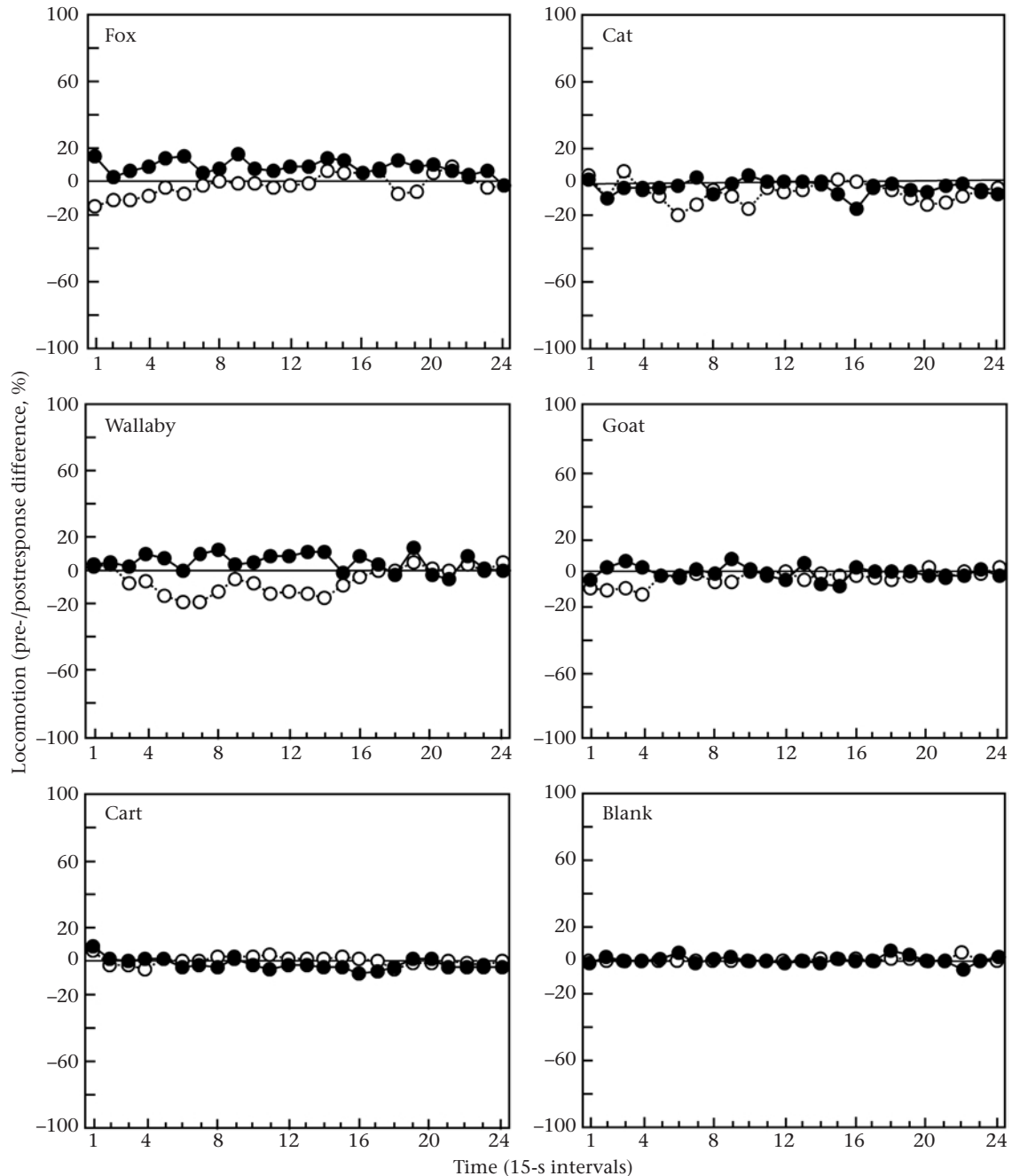


Figure 5. Changes in locomotion after training for the paired-experimental (\bullet , $N=8$) and unpaired-control (\circ , $N=8$) groups. Mean differences have been calculated in the same way as for Fig. 3.

suggest that the probability of learning may be sensitive to details of the training regime adopted.

In pairwise comparisons between stimuli, levels of relaxed behaviour in the presence of the fox were significantly different only to those during the blank control (Table 2). However, the effect sizes calculated for the fox-goat and the fox-cart comparisons (Table 2) were also large (Cohen 1988), suggesting that failure to detect significant differences in these comparisons probably reflects our small sample size (eight individuals per treatment) which provided only limited statistical power. For

ethical reasons, we deliberately used a minimal number of animals; this was nevertheless sufficient to reveal robust paired versus unpaired differences for several stimuli (Table 1). In contrast, we obtained a small effect size for the fox-cat comparison (Table 2), suggesting that increased sample size would not have revealed a reliable difference. We also obtained a small effect size for the Group \times Stimulus term in the fox-wallaby comparison. However, differences in the responses evoked by the fox and wallaby models are apparent in the highly significant Group \times Stimulus \times Time interaction for these two

stimuli (Table 2) which reflects the relatively transient change in behaviour to the wallaby (Fig. 3).

Our experimental design ensured that any differential change in behaviour between pre- and post-training trials could be attributed to the animals' intervening experience. Subjects in one group had the opportunity to associate a model fox with an aversive event, while those in the other had identical experience, but without this predictive relationship (Fig. 2). Changes in response to the cat thus necessarily reflect generalization from the animals' experience of the fox during training. The pattern of responses evoked by the fox and cat models was consistent with results obtained in classical studies of stimulus generalization (reviewed by Shettleworth 1998), which describe a gradual decrease in overall response intensity as the physical properties of the test stimulus differ progressively more from those of the stimulus with which training was conducted. In our study, the fox evoked the strongest fear response, which was characterized by a brief immediate increase in locomotion (Fig. 5), followed by prolonged vigilance (Fig. 4). Experimental animals responded somewhat less to the cat, but they nevertheless became more wary, which was reflected in a prolonged increase in vigilance (Fig. 4). The difference between these two patterns of responses shows that animals were able to discriminate the novel predator from the model with which they were trained, solely on the basis of morphological differences. Our results are consistent with Kramer & von St Paul's (1951) classic study of acquired predator recognition in bullfinches, *Pyrrhula pyrrhula*. These authors showed that hand-reared birds conditioned to respond fearfully to one species of kingfisher generalized their acquired response to other kingfisher species.

Generalization of the fear response from the fox to the cat must have been mediated by visual features common to both stimuli (Blumstein et al. 2000). All of our vertebrate models were of similar size and quadrupedal, so this effect presumably reflects characteristics shared only by the two predators. Convergent morphological features (e.g. frontally placed eyes), which are characteristics of many carnivores (Blumstein et al. 2000), are one obvious candidate, but additional experiments will be required to elucidate the precise basis of response generalization with our stimulus set. It will also be important to determine whether training with a live predator (e.g. McLean et al. 2000) produces similar effects.

Experimental animals maintained heightened responses to the fox and the cat throughout the post-training trials (Figs 3 and 4), while they resumed foraging, grooming and sitting within a few minutes after each training trial. This difference in response duration probably reflects cues present in the training regime: when the human exited the yard, this signalled that the capture attempt was over. In contrast, during post-training trials where no human appeared, wallabies probably remained vigilant because this predicted event had not occurred.

Surprisingly, the experimental group suppressed foraging and increased vigilance in response to the conspecific wallaby after training, although this change in behaviour was briefer than that evoked by the two predator models

and was not observed in the unpaired group. We cannot exclude the possibility that wallabies generalized their acquired fear response from the fox to the conspecific model. However, given the absence of such an effect with the goat, and the ephemeral nature of the response to the wallaby, we think this unlikely. Tamar wallabies are a moderately social species of macropod that tends to aggregate at food sources (Croft 1989), and we have found that conspecifics play an important role in the assessment of predation risk (Blumstein et al. 1999, 2000). The model wallaby was in a quadrupedal vigilant stance. We speculate that social cues became more salient in a 'risky' environment, such that the increased vigilance response of the paired subjects was socially facilitated (Zajonc 1969; Galef 1988). This possibility raises the interesting question of whether social companions can be a source of information about predation risk in tammars, which would be worthy of investigation in future studies.

Previous results from our work on wallaby antipredator behaviour have shown that captive tammars suppress foraging and increase vigilance in response to experimental presentations of both fox and cat models (Blumstein et al. 2000). These results, together with the selective learning apparent in the present study, strongly suggest that even predator-naïve tamar wallabies have retained the ability to discriminate predators from other vertebrates. Coss (1999) has provided additional examples of this phenomenon.

Many animals are predisposed to acquire certain types of information preferentially (Gould & Marler 1987). For example, Curio (1988) has shown that blackbirds learn to mob a species of bird to which they were initially indifferent (Australian honeyeater, *Philemon corniculatus*) once they have seen conspecifics apparently mobbing it. When the model bird was replaced with an arbitrary object (plastic bottle), the magnitude of the acquired response was much reduced. Similarly, Mineka & Cook (1988) showed that juvenile rhesus monkeys, *Macaca mulatta*, learned to be fearful of snakes by watching videorecordings of adults responding fearfully towards them, but they did not acquire a fear response when this same sequence was paired with a control stimulus (a bunch of flowers). These studies show that acquired responses can be less intense, or absent altogether, when the predator model is replaced by an arbitrary stimulus.

We have previously predicted that predator recognition training is more likely to succeed if the subject species already expresses some components of antipredator behaviour because training can then recover or enhance pre-existing elements (Griffin et al. 2000). Tamar wallabies from areas historically free of mammalian predators suppress foraging and watch model foxes and cats more than a cart or a blank presentation. In addition, these individuals are more likely to thump their hind feet in alarm in response to a fox than to other vertebrate models (Blumstein et al. 2000). Even predator-naïve tammars hence have some pre-existing antipredator behaviour system. Our results suggest that simple associative conditioning techniques may engage this system, raising the possibility of preferential learning about predator stimuli. Additional experiments, which will include

pairing nonpredator control stimuli with simulated capture, are planned to test this idea.

McLean et al. (2000) have shown that quokkas, *Setonix brachyurus*, hide from a model fox after undergoing a training procedure that involved being chased by a dog and suggest that this reflects generalization of acquired fear. It is possible that the quokkas used visual features shared by dogs and foxes to respond to both predators. However, this study did not quantify responses to a model nonpredator, which leaves the issue of response specificity unclear. Our results support McLean et al.'s (2000) suggestion that generalization occurs in training regimes of this kind. Furthermore, they provide the first evidence that generalization may be limited to predatory visual stimuli. We suggest that tamar wallabies may perceive predators as a natural category (Marler 1982). This cognitive property should enhance the probability of success in prerelease training programmes.

Our study was designed to explore the specificity of learning in a predator recognition context. This necessarily required a high level of experimental control, which we achieved by using taxidermic mounts presented in a standard way. Our results show that training inculcated a fear response to a model fox that combined immediate locomotion and sustained vigilance. Wallabies generalized this acquired wariness to another predator with which they had no experience during training. Whether such responses would also be expressed in response to a live predator, and whether they will be sufficient to enhance survival in the wild, are empirical questions that have yet to be addressed (but see van Heezik et al. 1999). Our results extend previous studies of learning in marsupials (reviewed by Wynne & McLean 1999) and raise unexplored issues concerning stimulus categorization and learning predispositions in these mammals. Furthermore, they are encouraging for those attempting to develop training techniques to help captive-bred individuals of endangered species survive encounters with potential predators after release. The challenge will be to initiate experimental reintroductions designed to evaluate the survival benefits of acquired behaviour.

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