

Innate or learned acoustic recognition of avian predators in rodents?

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SUMMARY

Calls of avian predators potentially predict danger for murine rodents. Indeed, exposure of field-experienced rodents to owl calls induces defensive behavior suggesting that recognition of vocalizations of avian predators is innate. To address this hypothesis, we investigated whether laboratory-reared and predator-naïve rodents (mice, gerbils, rats) express defensive behavior in response to calls of different avian predators but we observed no such defensive behavior. We then asked whether the calls of avian predators are faster or better learned as a danger-predicting cue than the calls of avian non-predators. All calls could be learned as danger-predicting cues, but we found no differences in the speed or strength of the learning. Taken together, our results suggest that there is no innate recognition of the calls of avian predators in murine rodents and that the recognition of the calls of avian predators observed in field-experienced rodents is acquired by learning.

Key words: anxiety, bird calls, fear conditioning, innate fear, predation, predator recognition.

INTRODUCTION

Anti-predator adaptations range from morphological structures like spines and armors to behavioral defensive responses such as avoidance behavior, increased vigilance, hiding and fleeing (Apfelbach et al., 2005; Blanchard and Blanchard, 1989a; Kats and Dill, 1998). Several studies have shown that recognition of predator-specific sensory cues is at least partly innate. Innate recognition of predator olfactory or visual cues has been shown for a diverse range of taxa, including fish (Hawkins et al., 2004), reptiles (Amo et al., 2005; Balderas-Valdivia and Ramirez-Bautista, 2005), birds (Göth, 2004), rodents (Apfelbach et al., 2005) and primates (Brown et al., 1992). Interestingly, this innate recognition of predator-related chemical or visual cues is even maintained in laboratory strains of rats and mice that were not exposed to selection pressure from predators for several hundred generations (Blanchard and Blanchard, 1990; Fendt, 2006; Guimaraes-Costa et al., 2007; Wallace and Rosen, 2000; Yang et al., 2004). As one example, our laboratory recently showed that urine samples of feline and canid predators but not of herbivore non-predators induce different species-specific defensive behaviors in predator-naïve, laboratory-reared rats (Fendt, 2006).

By contrast, with the exception of some indications in primates (Fichtel and van Schaik, 2006; Macedonia and Yount, 1991), there is little information available on a potential innate component of predator-related acoustic cues. Several authors discuss the possibility that murine rodents innately recognize calls of avian predators (Abramsky et al., 1996; Eilam et al., 1999; Hendrie, 1991; Hendrie et al., 1996; Hendrie et al., 1998; Hendrie and Neill, 1991). This could conceivably be adaptive, as owls and birds of prey exert a massive predation pressure on many rodent species. Experimental manipulation of the presence of avian predators affects aspects of rodent population dynamics. For example, the exclusion of predators

increased the population growth and peak size of the African pest rodent *Mastomys natalensis* (Vibe-Petersen et al., 2006). Murine rodents (e.g. mice, rats and gerbils) are all quite intensively hunted by avian predators and are one of the most important components of the diets of avian predators (Alivizatos et al., 2005; Charter et al., 2007; Dalbeck, 1996; Jedrzejewski and Jedrzejewska, 1993; Leonardi and Dell'Arte, 2006; Marchesi et al., 2006; Martinez and Lopez, 1999; Smal, 1989; Sommer et al., 2005; Tornberg and Colpaert, 2001). For example, rats can represent between 13 and 63% of the prey biomass of goshawks and owls (Alivizatos et al., 2005; Dalbeck, 1996; Martinez and Lopez, 1999; Tornberg and Colpaert, 2001), mice between 33 and 40% of the prey biomass of owls (Charter et al., 2007; Sommer et al., 2005). There are only a few reports on the predatory pressure of avian predators on gerbils. However, one study found that gerbils made up 15% of the prey biomass of owls (Leonardi and Dell'Arte, 2006). This overall high predation pressure by avian predators surely constitutes a selection pressure on rodents for the ability to recognize the presence of avian predators and to initiate appropriate behaviors that decrease the probability of being preyed upon (Eilam, 2005). Indeed, rodents can assess ambient predation pressure from avian predators and adapt their foraging behavior accordingly by visiting covered food patches more frequently than exposed ones (Mohr et al., 2003).

There remains the question of which cues rodents use to assess the presence of raptors. It is questionable whether the calls of avian predators are good indicators of immediate danger for murine rodents since avian predators vocalize *before* but not *during* hunting (cf. Hendrie et al., 1998). Their calls are mainly used for territorial defense and mate attraction. That is, calling avian predators are not an immediate threat. However, the number of calling birds surely correlates well with general ambient predation pressure. Induction of long-lasting defensive behaviors in prey animals, such as

increased vigilance and less exploring (cf. Abramsky et al., 1996; Eilam et al., 1999; Hendrie et al., 1998) would thus appear adaptive. Likewise, odors from predator excrements and territorial markings are indicators of a generally increased danger rather than an acute danger. Hence, odor-induced defensive behaviors mainly consist of risk assessment behavior and avoidance behavior but to a lesser extent freezing or flight behaviors [cf. discussion in Fendt (Fendt, 2006)]. For predator calls, a recent field study indeed showed that owl playbacks during full moon dramatically reduce the spatial activity of a prey rodent (Schmidt, 2006). Several laboratory studies (Abramsky et al., 1996; Eilam et al., 1999; Hendrie, 1991; Hendrie et al., 1994; Hendrie et al., 1996; Hendrie et al., 1998; Kotler et al., 1992) further demonstrate that the calls of owls, the main nocturnal predators of murine rodents, are able to induce physiological and behavioral signs of fear in different murine rodents. Enhanced stress hormone levels (Eilam et al., 1999) and increased analgesia (Hendrie, 1991), two physiological signs of fear, were measured during exposure to owl calls. In the latter study, laboratory mice (strain DBA/2) were the experimental subjects, whereas in the other study wild murine rodents [social Gunther's voles (*Microtus guentheri* Danford and Alston 1880) and common spiny mice (*Acomys cahirinus* Desmarest 1819)] were tested. In these and other wild rodents [social voles (*Microtus socialis* Pallas 1773), dormice (*Eliomys melanurus* Wagner 1840), jerboas (*Jaculus jaculus* Linné 1758) and different gerbil species (*Gerbillus allenbyi* Thomas 1918; *Gerbillus pyramidum* Geoffroy 1825)] behavioral signs of fear were also induced by the calls of avian predators. For example, crouched freezing, one of the most prominent behavioral signs of fear in rodents, was observed in different rodent species during (but not after) the exposure to owl calls (Eilam et al., 1999; Hendrie et al., 1998). In laboratory-reared rodents (DBA/2 mice), such defensive behaviors to predator bird calls were not observed in untreated predator-naïve mice (Hendrie et al., 1996; but see also Hendrie and Neill, 1991). Only after treatment with alprazolam, an anxiolytic benzodiazepine, was an increase in crouched freezing during and after exposure to owl calls found (Hendrie et al., 1996). The authors suggested that after alprazolam treatment, 'protean behavior is replaced by more organized vigilance behaviors'. Taken together, behavioral responses to the calls of avian predators were only observed in wild-caught, field-experienced rodents or pharmacologically treated laboratory mice. Therefore, the question of whether the recognition of these calls was learned or at least partly innate remained unanswered.

The aim of the present study was to fill this gap by testing whether there is an innate fear response of laboratory-reared and predator-naïve murine rodents during or after the exposure to calls of avian predators. The term 'avian predator' is used here to refer to both diurnal (Accipitiformes and Falconiformes) and nocturnal (Strigiformes) avian raptors. Here, we explicitly focus on acoustic predator cues, excluding cues in other sensory modalities as well as possible interactions of multiple cues. Our first experiment was based on the studies discussed above (Eilam et al., 1999; Hendrie and Neill, 1991; Hendrie et al., 1996; Hendrie et al., 1998) and had a very similar experimental procedure. We extended their studies by testing not only laboratory mice (*Mus musculus* Linné 1758) but also laboratory rats (*Rattus norvegicus* Berkenhout 1769) and pallid gerbils (*Gerbillus perpallidus* Setzer 1958). We tested for several behaviors which are affected by potentially threatening stimuli, such as freezing behavior, exploratory behavior, and vigilant rearing. The first behavior is typically increased during exposure to threatening stimuli, the latter two are typically decreased (e.g. Dielenberg et al., 2001). Since we found no specific behavioral effects of the exposure

to calls of avian predators *versus* non-predators (six different avian calls) in our animals, we concluded that the defensive response to avian calls could be a learned behavior. We then asked whether the calls of avian predators are faster or better learned as danger-predicting cues than the calls of non-predators. To mimic the association of bird vocalizations with an aversive experience such as an attack, we carried out two fear-conditioning experiments in rats. We used the calls of avian predators or non-predators as conditioned stimuli (CS) and electric foot shocks as unconditioned stimuli (US). In the first of these two experiments, complete fear conditionings with ten pairings of the CS and the US were carried out with three different avian calls to check whether fear conditioning to calls of avian predators or of avian non-predators is possible in general. Since we observed fear conditioning in this experiment and no differences regarding the used CS, we tested in a further experiment whether one-trial fear conditioning is possible, i.e. only one CS-US pairing was presented (nine different avian calls). If predator calls were (innately) more salient to rodents, one-trial learning might be sufficient for predator calls, but not for non-predator calls. The conditioning experiments associated an immediate danger (foot shock) with the bird calls, and hence, we this time tested for an immediate fear response behavior, i.e. freezing.

MATERIALS AND METHODS

Animals

Male C57/BL6J mice ($N=10$; Charles River GmbH, Sulzfeld, Germany) and male Sprague-Dawley rats ($N=130$; Charles River), as well as male pallid gerbils ($N=8$; *Gerbillus perpallidus*, bred at the Institute of Zoology, University of Tübingen) aged 2–4 months were used. All animals were maintained on a 12 h:12 h light:dark cycle, and food and water were available *ad libitum*. All animals were under regular veterinary control and germ-free. Animal care for all species followed standard protocols; all individuals of a given species were treated the same way by the same team of caretakers. Therefore, it is highly unlikely that differential individual histories, e.g. manipulation of cubs during cage cleaning and associated changes in neural plasticity, biased our behavioral data (Giovenardi et al., 2005) (c.f. Winkelmann-Duarte et al., 2007). The animals were tested during the light period. All experiments were performed in accordance with ethical guidelines for the use of animals in experiments and were approved by the local animal care committee (Regierungspräsidium Tübingen, ZP 4/02).

Acoustic stimuli

For the presentation of bird calls, digital recordings from the CD "Vogelstimmen Europas" (European bird calls; Jean C. Roché, Kosmos Verlag, Stuttgart, Germany) and recordings generously provided by Professor C. König (Staatliches Museum für Naturkunde, Stuttgart, Germany) were used. As predator calls, calls of the following birds were used in experiment 1: tawny owl (*Strix aluco* Linné 1758), barn owl (*Tyto alba* Scopoli 1769), peregrine falcon (*Falco peregrinus* Tunstall 1771). Calls of the following birds were used as non-predator calls: chaffinch (*Fringilla coelebs* Linné 1758), blackbird (*Turdus merula* Linné 1758) and mallard (*Anas platyrhynchos* Linné 1758). In experiment 2, only calls of the blackbird, tawny owl and eagle owl were used. In experiment 3, nine different calls were used. In addition to the calls of experiment 1 (except for calls of the peregrine falcon) calls of the eagle owl (*Bubo bubo* Linné 1758), goshawk (*Accipiter gentilis* Linné 1758) and kestrel (*Falco tinnunculus* Linné 1758) were used as predator bird calls, and quail (*Coturnix coturnix* Linné 1758) as a non-

predator bird call. Cool Edit (Syntrillium Software Company, Phoenix, AZ, USA) was used to upsample all recordings to 96 kHz, to generate the silence for pre-phase and post-phase, and to assemble the playback files.

Playbacks

Digital recordings of the bird calls were played back from a PC (sound card, Yamaha DS1x Native Audio, 48 kHz sampling rate D/A conversion), which was connected *via* an amplifier (WPA-600 Pro, Conrad, Hirschau, Germany) to a loudspeaker (SP-200X, Monacor, Bremen, Germany; flat frequency response (± 3 dB) between 200 Hz and 10 kHz). The loudspeaker was mounted over one corner of the arena in a height of 50 cm. The playbacks were presented at an intensity that the peak sound pressure level of the bird calls was between 70 and 80 dB SPL – measured in the centre of the test arena (mean calculated from the four highest amplitude peaks per playback sequence). Thus, calls were in a naturalistic range [no published measurements of source levels available for avian predators, but compare Brackenbury, Brumm and Nemeth (Brackenbury, 1979; Brumm, 2004; Nemeth, 2004)] and above hearing threshold for all three rodent species tested [mice (Ehret, 1976; Ison et al., 2007); rats (Kelly and Masterton, 1977); gerbils (Ryan, 1976)]. Measurements of sound pressure level were obtained with a quarter-inch (0.635 cm) condenser microphone (Brüel and Kjaer, Copenhagen, Denmark), a custom-build D/A converter (PCTape, Department of Animal Physiology, University of Tübingen; 96 kHz sampling rate, 16 bit, digital anti-aliasing) and software (Selena, Department of Animal Physiology, University of Tübingen) [for details on the software see Siemers and Kerth (Siemers and Kerth, 2006)].

Apparatus

All behavioral tests were conducted in a square arena with walls made from transparent Plexiglas (100 cm \times 100 cm; custom-made at the University of Tübingen). During the tests, the arena was dimly illuminated by red light (60 W red bulb, ~ 5 lux) to mimic the crepuscular conditions during the onset of the animals' natural activity period. The arena was located in a sound-attenuated measurement chamber (3.3 m \times 3.3 m \times 2.2 m). All animals that were not currently being tested were housed outside the chamber so they could not overhear the calls presented to their conspecifics.

Pavlovian fear conditioning in experiments 2 and 3 was conducted in a small conditioning box (40 cm \times 60 cm \times 30 cm), likewise located in the sound-attenuated measurement chamber. The box was made of PVC, the floor of the box consisted of stainless steel bars. The conditioned stimulus was the playback of a bird call recording (see above) presented *via* a loudspeaker which was mounted in the lid of the conditioning box. The unconditioned stimulus was a 0.6 mA foot shock delivered through the floor bars during the last 0.5 s of presentation of the bird call recording.

Behavioral analysis

The behavior of the animals was videotaped for documentation and simultaneously displayed on a monitor for online analysis by the experimenter. The duration of the following behaviors were quantified with event-recorder software (custom-made, University of Tübingen). (1) Freezing, defined as a cessation of all movements except those which are necessary for breathing. Freezing is a well-studied, specific defensive behavior in rodents (Blanchard and Blanchard, 1969). (2) Exploratory behavior. (3) Vigilant rearing, as a measure for vigilance (Dielenberg et al., 2001). Changes in the prevalence of all three behaviors have previously been shown

to indicate a state of fear in rodents. Perception of potential danger will induce fear, which in turn elicits freezing as an acute defensive response. An increase of vigilant rearing and a reduction of exploratory behavior are believed to be more long-lasting adaptive changes to danger (Dielenberg et al., 2001; Fendt and Fanselow, 1999; Misslin, 2003; Yamada and Nabeshima, 1995). In experiment 1, we therefore used these three measures to assess whether calls of different bird species induce fear behavior on a behaviorally relevant level in any of the three rodent species tested. In the two other experiments, we focused our analysis on freezing. The reason for this is that in experiments 2 and 3 we associated an aversive stimulus (foot shock), i.e. immediate danger, with the bird calls and hence freezing as an immediate fear response was an appropriate behavioral variable to quantify (Fendt and Fanselow, 1999).

Experimental procedures

Experiment 1 (unconditioned fear in response to predator bird calls)

To familiarize the animals ($N=8-10$ of each species) with the test arena, each animal was placed into the arena for 10 min once per day on three consecutive days. On the fourth day, the actual experiment started and the response of the animals to different bird calls was measured in the same test arena. Subjects were released and presented with a 10 min sound file playback. The first 2 min contained only silence without any stimuli (pre-phase), then bird calls were presented for 3 min (stimulus phase) and finally there were 5 min of silence again (post-phase). The behavior of the animals was observed and analyzed for the whole 10 min. On the following days, each animal was tested once per day between 8:00 h and 16:00 h with one of the bird calls. The different bird calls were presented in a pseudorandomized order.

Experiment 2 (fear conditioning to predator bird calls with 10 pairings)

After familiarization to the test arena (see above), the rats ($N=10$ for the each bird call) were tested for an unconditioned response to the bird call (pre-test). One day later, the animals were put into the conditioning box, and after 3 min without any stimulation, the respective bird call was played back for 2 min. During the last 0.5 s of the playback, a foot shock was administered *via* the floor grid. Thereafter, nine further pairings of the bird call and the foot shock were presented. The interval between these presentations was between 5.5 and 8.5 min (mean: 7 min). Two days later, the animal was put into the test arena and was tested for a conditioned response to the bird call (post-test). The procedure for the pre- and post-test was exactly the same as in experiment 1.

Experiment 3 (one trial fear conditioning)

The experimental procedure was exactly the same as described in experiment 2 ($N=10$ for each bird call) except that only one pairing of the bird call and the foot shock was presented. Thereafter, another 5 min elapsed without stimulation and the animal was put back into the home cage. Seven hours later, the animal was put into the test arena and was tested for a conditioned response to the bird call (see above, post-test). Again, the procedure for the pre- and post-test was exactly the same as in experiments 1 and 2.

Statistical analysis

For statistical analysis, the mean durations of the different behaviors per minute in the different test phases (pre-phase, stimulus phase, and post-phase) were calculated. Both the mean

response to the calls of all predator *versus* non-predator birds as well as the mean response to the calls of any single bird species were analyzed. To do this, we carried out paired *t*-tests or repeated-measure ANOVAs with the factor 'bird call' (either grouped as predator bird *vs* non-predator bird calls or else each specific bird call) separately for each phase. 'Bird call' was a within-subject factor in experiment 1, and a between-subject factor in experiments 2 and 3. In later experiments, the factor 'test day' (naive *vs* conditioned) was additionally used as a within-subject factor. All tests were calculated using Systat 10. To estimate whether our sample size is large enough to detect biologically relevant changes in the observed behavior, we calculated separate post-hoc power analyses for each group of animals and each behavior with the values (mean, standard derivations) of the pre-phase (naive behavior). For freezing behavior, which was in the focus of our study, we can detect changes of $\pm 12\%$ with our sample sizes (all $N=10$) and a statistical power of $\beta=0.80$.

RESULTS

Experiment 1: unconditioned fear

Mice

Ten C57/BL6J mice were tested for unconditioned effects of predator and non-predator bird calls. Separate repeated-measure ANOVAs for each phase of the tests (pre-phase, stimulus phase, post-phase) with the different observed behaviors as dependent factors and the call type (non-predator *versus* predator) as independent factors revealed no influence of the call type on the behavior of the mice in each test phase (for all tests, $F < 1.69$, $P > 0.2$ for freezing, exploratory behavior and vigilant rearing; Fig. 1). We further tested whether calls of any specific bird species affected the mice's behavior more strongly than those of other bird species. However, there were also no significant differences among exposures to different species' calls for freezing, exploratory behavior or vigilant rearing (repeated-measure ANOVAs: for all tests, $F < 1.88$, $P > 0.12$).

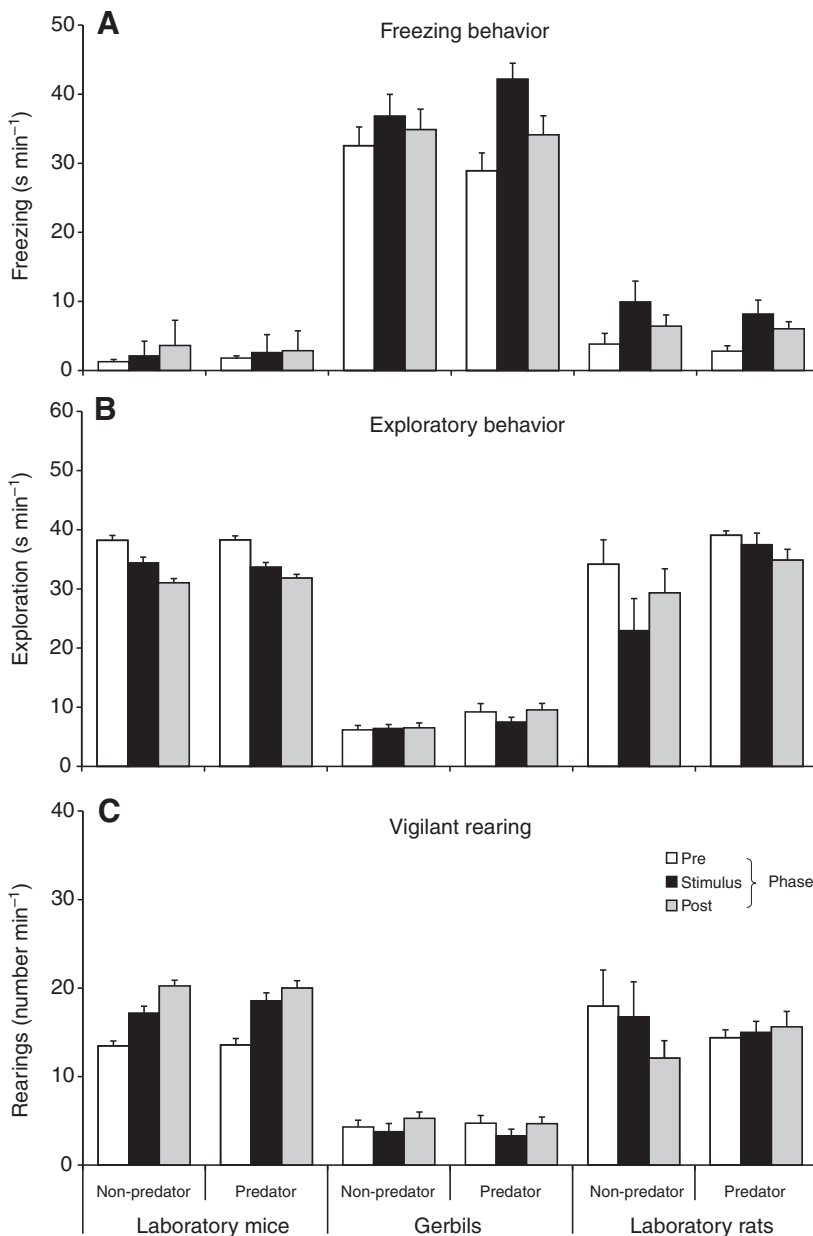


Fig. 1. Unconditioned effects of non-predatory and predatory bird calls in mice, gerbils and rats. (A) The freezing behavior (seconds per minute), (B) the exploratory behavior (seconds per minute) and (C) the number of vigilant rearings of each phase; values are means, with bars indicating the standard errors of the mean (s.e.m.). There were no significant effects of the different bird calls on the different behaviors.

Gerbils

Eight gerbils were tested for the effects of predator and non-predator bird calls. Freezing, exploratory behavior and vigilant rearing were not different in the test phases of the exposure to avian predator and non-predator calls (repeated-measure ANOVA: for all tests, $F < 2.69$, $P > 0.11$; Fig. 1). For exploratory behavior, we found an almost significant higher duration in the post-phase after exposure to avian predator calls ($F_{1,37} = 3.98$, $P = 0.055$), but in these test trials there was also a tendency for different levels of exploratory behavior in the pre-phase ($F_{1,37} = 2.38$, $P = 0.08$), indicating that this tendency is not specific to avian predator calls. This is supported by the analysis of the possible effects of the individual bird species, which also revealed no differences in the observed behaviors in any phases of the tests (repeated-measure ANOVAs: for all tests, $F < 1.16$, $P > 0.36$).

Rats

Ten rats were tested. Again, there were no significant differences in the freezing, exploratory behavior, and vigilant rearing of the animals in the phases before, during or after exposure to avian predator and non-predator calls (repeated-measure ANOVAs: for all tests, $F < 1.0$; Fig. 1). In addition, inspection of possible effects of the calls of individual bird species revealed no significant effects (repeated-measure ANOVAs: for all tests, $F < 1.13$, $P > 0.35$).

Experiment 2: fear conditioning (10 pairings)

In our fear-conditioning experiments, we focused our analysis on freezing, a well described typical response of rats to fearful stimuli (Blanchard and Blanchard, 1969; Fendt and Fanselow, 1999). We also quantified the other behaviors described in experiment 1 (data not shown). In general, a significant increase in freezing lead to a decrease of the other behaviors (exploratory behavior, vigilant rearing), whereas no change in freezing was also connected with no changes in the prevalence of the other behaviors.

After ten pairings of bird calls with aversive foot shocks, each group of animals ($N = 10$ for each of the three species of bird used as playbacks) showed increased freezing in each phase of the test after the conditioning training (repeated-measure ANOVAs, factor test day: for all tests, $F > 13.2$, $P < 0.002$; Fig. 2). Importantly, the level of freezing in the different phases was differently increased by the fear conditioning (interaction phases \times test day: for all tests, $F > 6.37$, $P < 0.003$), with largest effects during and

after bird call presentation, indicating that the fear conditioning was specific to the bird call. It is important to note that there were no differences between the calls of the three bird species in the amount of conditioning-induced additional freezing, i.e. in the effectiveness of conditioning (overall ANOVA, interaction phases \times test day \times stimulus type: $F_{2,52} < 1.0$).

Experiment 3: one-trial fear conditioning

For the pooled data sets for both avian predator and non-predator calls, after one single pairing of a call with an aversive stimulus, the amount of freezing in each phase before and after fear conditioning did not differ (repeated-measure ANOVAs: for all tests, $F < 1.30$, $P > 0.26$; Fig. 3A). That is, one-trial conditioning was by and large not sufficient to induce conditioned fear. When analyzed separately, the calls of some individual species were able to elicit one-trial fear conditioning (repeated-measure ANOVA: for all tests, $F > 2.89$, $P < 0.01$ in the stimulus and post-phase; Fig. 3B). Specifically, the calls of the predator species, tawny owl (*post-hoc* Tukey's tests against naive behavior: post-phase: $t = -4.25$, $P = 0.002$) and goshawk (stimulus phase: $t = 2.48$, $P = 0.035$) as well as of the non-predator mallards (post-phase: $t = 3.30$, $P = 0.009$) induced conditioned freezing after one pairing with an aversive foot shock (other $P > 0.1$).

DISCUSSION

Murine rodents are exposed to an enormous predatory pressure by avian predators (Alivizatos et al., 2005; Charter et al., 2007; Dalbeck, 1996; Jedrzejewski and Jedrzejewska, 1993; Leonardi and Dell'Arte, 2006; Marchesi et al., 2006; Martinez and Lopez, 1999; Smal, 1989; Sommer et al., 2005; Tornberg and Colpaert, 2001). Therefore, the hypothesis was proposed (Hendrie and Neill, 1991) that murine rodents can innately recognize avian predator calls, which then quickly and effectively induce defensive behavioral responses. The first aim of our study was to investigate whether there indeed is innate (unconditioned) fear behavior in murine rodents during or after exposure to predator bird calls. If murine rodents innately recognize the calls of avian predators, defensive behavior should be elicited by predator bird calls but not by non-predator bird calls. That is, in our experimental design, an increase in freezing behavior and vigilant rearing [more immediate defensive behavior (Blanchard and Blanchard, 1969; Dielenberg et al., 2001; Eilam et al., 1999; Fendt and Fanselow, 1999; Hendrie et al., 1998)]

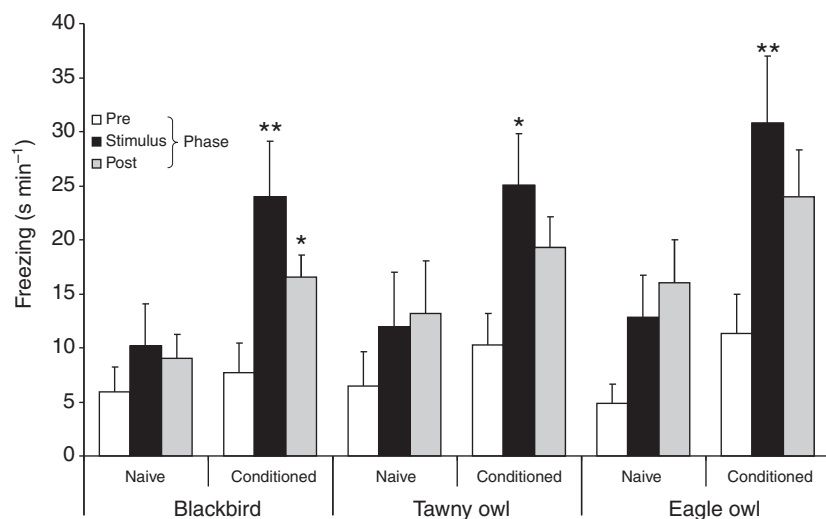


Fig. 2. Freezing behavior to bird calls after fear conditioning (10 trials, rats). Values are the mean freezing behavior (seconds per minute) of each phase; bars indicate the standard errors of the mean (s.e.m.). In each group of animals, there was an increase of freezing behavior after fear conditioning to the respective bird call. ** $P < 0.01$, * $P < 0.05$ *post-hoc* Tukey's test (comparison with naive behavior) after an ANOVA.

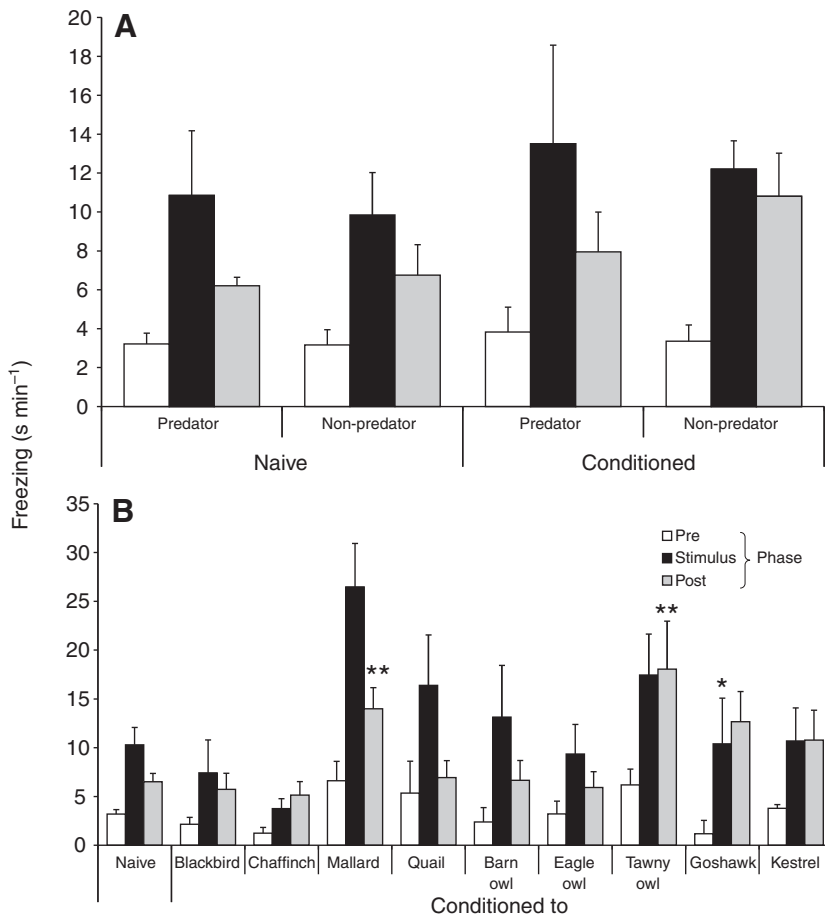


Fig. 3. Freezing behavior to bird calls after one-trial fear conditioning (rats). Values are the mean freezing behavior (seconds per minute) of each phase; bars indicate standard errors of the mean (s.e.m.). (A) Data grouped for predator and non-predator calls: there was no increased freezing in response to predatory bird calls compared with non-predatory bird calls. (B) Data classified for single calls: analysis of the single 'species' calls revealed significant effects of some bird calls after one-trial fear conditioning (mallard, goshawk, tawny owl). Please note that for this figure, the unconditioned behavior to the different bird calls were pooled whereas for the statistical analysis, a comparison within each group of animals were made (pre- vs post-conditioning) $**P < 0.01$, $*P < 0.05$ *post-hoc* Tukey's test (comparison with naive behavior) after an ANOVA.

as well as an inhibition of exploratory behavior [more long-lasting defensive behavior (Misslin, 2003; Yamada and Nabeshima, 1995)] should occur during or after the presentation of avian predator calls but not of non-predator calls.

For all three species (C57/BL6J mice, Sprague-Dawley rats, pallid gerbils) our laboratory-reared and predator-naive subjects did not show any decrease in exploratory behavior or increase in freezing behavior or vigilant rearing during or after exposure to predator bird calls compared with exposure to non-predator bird calls. It is important to note that the sample size we took is large enough to detect biologically relevant changes in behavior (see power analysis, as well as results from experiments 2 and 3) and that the type of apparatus used (medium-sized open field without shelter) is shown to support freezing as a defensive behavior (Blanchard and Blanchard, 1989a; Blanchard and Blanchard, 1989b; Griebel et al., 1995a; Griebel et al., 1995b). Furthermore, all acoustic stimuli used (frequency range: 0.3–9 kHz; intensity: 70–80 dB SPL) were clearly perceptible for the murine rodents we worked with (cf. audiograms cited in the Materials and Methods section). This also applies for the C57/BL6J mice which are known to suffer from a hearing deficit, mainly at high frequencies at an older age (e.g. Ison et al., 2007), but the animals we used were young, non-hearing-impaired animals. Successful fear conditioning in rats (experiment 2) is further evidence that the calls were well audible at the amplitudes presented. Taken together, these results clearly support the study by Hendrie et al. (Hendrie et al., 1996) that also showed no behavioral signs of fear in laboratory-reared mice upon exposure to avian predator calls. Only after a pharmacological treatment with alprazolam, did Hendrie et al. (Hendrie et al., 1996) find call-induced fear. We refrained

from repeating such a treatment, because we argue that a biologically meaningful innate recognition of a predator cue should induce appropriate avoidance reactions and behavioral signs of fear without experimental drug administration. Furthermore, it is not apparent why treatment with an anxiolytic drug such as alprazolam should induce more defensive behavior (i.e. anxiogenic-like effects) in such an experiment.

It is remarkable that gerbils expressed much more freezing behavior than mice and rats. Since there are no published studies on fear behavior in Pallid gerbils, we do not know whether this reflects the normal behavior of this species. However, this species has been bred for a much shorter time in captivity than rats and mice (~10–15 years vs ~100 years), and the higher freezing response might reflect this.

As outlined above, it can be conjectured that an innate recognition of avian predator calls would be an enormous evolutionary advantage for murine rodents. Since owls are key nocturnal predators for many species (Dalbeck, 1996; Jedrzejewski and Jedrzejewska, 1993; Mebs and Scherzinger, 2000) a long-lasting increase in defensive behavior after hearing an owl call could have a large impact on the survival of the rodent. One possible defensive behavior is freezing, which leads to a suppression of all noises induced by the rodent's movements. Since owls mainly localize prey animals acoustically [summarized by Gaese and Wagner and by Konishi (Gaese and Wagner, 2002; Konishi, 2003)], this would effectively preclude sensory access of the owls to their prey. In fact, freezing behavior is one of the preferred defensive behaviors during encounter with an avian predator (Edut and Eilam, 2004; Eilam, 2005). If the owl had already heard the rodent rustling, freezing

might be a suboptimal strategy, because the owl could still find its prey by searching the location where the last sound came from (Eilam, 2005). However, as owls call mainly for territorial defense or intra-pair communication (e.g. Penteriani, 2002) but never during hunting, hearing a call does not indicate an imminent attack. Hence, increased freezing and decreased production of rustling sounds might not be an appropriate reaction [c.f. discussion in Hendrie et al. (Hendrie et al., 1998)]. During the day, raptors are predators of many murine rodents (Tornberg and Colpaert, 2001). Raptors use their visual system for hunting and are extremely sensitive to prey motion. So, freezing behavior during or after a raptor call could decrease the chance of detection by an avian predator. Again, freezing behavior is not very reasonable if a prey animal has already been detected by the predator or if an escape route or a refuge is available. This dilemma is discussed in a review article by David Eilam (Eilam, 2005) and he called it "A time to run, a time to freeze". For example, a dangerous stimulus which is discrete and well localizable (e.g. a visible predator) will more likely promote flight behavior, whereas a stimulus which is difficult or amorphous to locate (e.g. a predator call or odor, particularly presented in a small arena without shelter) will more likely promote freezing behavior (Eilam, 2005). Although the academic debate on the adaptive value of freezing is ongoing, empirical data clearly demonstrate that rodents do increase freezing in response to calls of avian predators or during encounters with avian predators (Edut and Eilam, 2004; Eilam et al., 1999; Eilam, 2005; Hendrie et al., 1998). The fact that we did not observe such an increase in freezing in laboratory-reared, predator-naïve rodents argues against an innate basis of this recognition-triggered behavioral response.

The first experiment clearly indicated that there is no innate fear behavior in response to predator bird calls. The second experiment showed that this lack of fear behavior was not due to sensory bias since fear conditioning to the calls was possible, i.e. the calls were clearly perceptible by the rodents used in the experiments. We then went one step back to test the hypothesis that murine rodents might possess an innate predisposition to acquire learned fear faster in connection with calls of avian predators than with non-predator calls. We used a one-trial fear conditioning paradigm to assess whether rats are able to associate danger faster with predator calls than with non-predator calls. One-trial learning has been described for several learning phenomena with high ecological relevance [e.g. aversive tastes (Servedio, 2000)]. Therefore, we believed that testing for one-trial conditioning would be a suitable paradigm for assessing a predisposition for quick learning (i.e. a prepared learning process). For example, a previous study of our laboratory demonstrated that fear conditioning experiments are indeed able to identify prepared learning of biologically relevant acoustic stimuli in rats (Endres et al., 2007).

However, our one-trial fear conditioning experiment in rats (experiment 3) demonstrated that, in general, neither predator nor non-predator bird calls were learned as a conditioned fear stimulus after only one negative experience. We conclude that laboratory rats have neither an innate recognition of predator bird calls nor a specific predisposition ('preparedness') for a fast learning of predator bird calls as danger-predicting cues. However, laboratory rats have been bred in captivity for more than 100 years (i.e. over 300 generations) and it could be possible that both innate fear to predator bird calls and an innate predisposition for a fast fear conditioning of these calls have been lost during this time. This scenario is rendered less likely by the interesting fact that innate recognition of danger-predicting cues with other modalities is still present in rats (e.g. predator odors, cf. Introduction).

Although, in general, neither predator nor non-predator bird calls were learned as a fear conditioned stimulus in our one trial experiment, we did observe successful one-trial conditioning with tawny owl, goshawk and mallard calls. While tawny owls and goshawks prey on rats (Alivizatos et al., 2005; Martinez and Lopez, 1999; Tornberg and Colpaert, 2001), mallards certainly do not. We compared the playback sequences from these three species with the other playbacks we had used to identify potential features that would unify them or make them especially conspicuous to the rats, but we did not find any clear pattern that would separate them from all other playbacks used. Although the tawny owl playbacks were nearly constant frequency calls with peak frequencies <1 kHz, the other two species showed considerably more frequency modulation, broadband elements, peak frequencies around 2 kHz and also higher duty cycle (more broadcast signal per unit time). All three species' calls were certainly less melodic than those of the passerine songbirds in our sample, but this also holds for the other owls that did not elicit one-trial fear conditioning. The acoustic diversity of the calls of avian predators both within and among species and the overlap in call parameters with non-predator birds might render the genetic fixation of a simple, generalized, raptor-specific call template difficult in a rodent species. It might prove more efficient to learn the calls of raptor species that locally co-occur with the respective rodent population. Non-lethal trait-mediated effects, where behavioral compensation to predation risk occurs, such as animals avoiding areas of high predation risk may be larger than lethal effects in determining the behavior, condition, density and distribution of animals (Cresswell, 2008). Such cost associated with excessive vigilance and 'false alarm' antipredator behavior will provide additional selection pressure that favors behavioral plasticity and learning of predator cues over innate responses. As predator attacks on rodents are very often fatal (Curio, 1976) and hence leave little room for the prey to learn to avoid future attacks, we presume that social learning plays a crucial role in this process.

In contrast to calls in birds, chemical cues in mammals allow for a much more reliable discrimination between predatory and non-predatory species (Apfelbach et al., 2005; Fendt, 2006). This is due to the direct origin of these odor components from metabolizing meat (Berton et al., 1998). We assume that this more reliable chemical dichotomy has supported the evolution of innate olfactory recognition of predatory mammals in rodents as opposed to a more flexible, probably learning-based recognition of avian predator calls.

In summary, our experiments demonstrate that there is neither innate (unlearned) fear specific to predator bird calls nor a predisposition to associate such calls with danger faster than non-predator calls. The first was observed in three different laboratory-reared murine rodent species, whereas the latter was only tested in rats. Although there seems to be no innate recognition of avian predator calls, wild-captured or free-living murine rodents do show defensive behavior upon exposure to predator bird calls (Abramsky et al., 1996; Eilam et al., 1999; Hendrie et al., 1998; Kotler et al., 1992; Schmidt, 2006). Taken together, this suggests that wild rodents socially learn the danger-predicting meaning of predator bird calls during infancy and adolescence. Social learning is discussed elsewhere in the context of anti-predator strategies (Galef and Laland, 2005; Griffin and Galef, 2005). Whether it indeed mediates recognition of the calls of avian predators has to be addressed by future studies.

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