



Parental Alarm Calls of the White-Crowned Sparrow Fail to Stimulate Corticosterone Production in Nest-Bound Offspring

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Abstract

Alarm calling by parents is widespread among animals and has strong implications for parent and offspring fitness, yet it is virtually unknown whether parental alarm calls can initiate a corticosterone response in offspring. We investigated whether parental alarm calls of the white-crowned sparrow, *Zonotrichia leucophrys*, activated the corticosterone response of their nest-bound young, as such a response might prepare older nestlings for premature fledging and increase their survival when contacted by a predator at the nest. We conducted an experiment in which nestlings were either exposed to parent alarm calls (treatment) or experienced a period without parental alarm calls (control) immediately prior to blood sampling. We then sampled nestlings to measure corticosterone levels within 4 min of first contact (baseline corticosterone) and 60 min later (handling-induced corticosterone). Young nestlings (i.e. 3–4 d post-hatch) did not exhibit a corticosterone response to parental alarm calls or to handling, as mean corticosterone levels were similar in the control and treatment groups for both baseline and 60-min post-baseline samples. Against our predictions, there was no difference in mean levels of baseline corticosterone between control and treatment groups in older nestlings (i.e. 7–8 d post-hatch) that were capable of surviving out of the nest. However, we did find a significant increase in mean levels of corticosterone after handling in both groups, which indicated that older nestlings were able to mount a functional corticosterone response when confronted with a potential predator. Why older nestlings did not initiate a corticosterone response after exposure to parental alarm calls is unclear but may have occurred because the costs of mounting such a response outweighed the benefits, perhaps because of growth or developmental costs.

Introduction

In birds, the release of corticosterone by the adrenal glands is an important physiological response to an environmental stressor (Sapolsky et al. 2000; Romero 2004). Rapid elevation of corticosterone induces an 'emergency life-history stage' that is characterized by physiological and behavioral changes that increase the likelihood of an individual's short-term survival

(Wingfield et al. 1998; Wingfield 2003; Martin 2009). Unpredictable encounters with a predator have been shown to elevate corticosterone levels and, in turn, induce individuals to undertake behaviors that maximize survival when encountering a predator. For example, after individual great tits (*Parus major*) were exposed to a stuffed owl in a laboratory setting, corticosterone levels increased as did the time individuals spent hiding in protective

cover (Cockrem & Silverin 2002). Similar results have been found in the pied flycatcher (*Ficedula hypoleuca*; Silverin 1998), the European stonechat (*Saxicola torquata rubicola*; Canoine et al. 2002), and the Tanzanian stonechat (*Saxicola t. axillaris*; Scheuerlein et al. 2001), suggesting that an elevation of corticosterone and an undertaking of behaviors that increase survival during exposure to predators are widespread and highly conserved among passerine birds.

Nest predation is a common source of nest failure for birds that build open-cup nests, with nest predation rates exceeding 50% in some species (Ricklefs 1969; Martin 1995). Such extensive predation has selected for a suite of traits in adults that minimize the likelihood that a nest is discovered by a predator. Many species construct nests that are cryptic and difficult to locate among available microhabitats (Martin 1993) and restrict conspicuous displays to areas away from the nest (but see Leonard 2008). For altricial and semi-altricial species that continue their development in the nest after hatching (Starck & Ricklefs 1998), offspring behaviors, such as begging, can increase the likelihood that predators will discover the nest (Leech & Leonard 1997; Briskie et al. 1999; Haskell 2002). In response to predators near their nest, adults of many altricial and semi-altricial species give alarm calls that can lead to behavioral responses in offspring that reduce their conspicuousness to predators. In many species, nestlings do not typically alter behaviors when exposed to parental alarm calls shortly after hatching, presumably because of reduced sensory modalities (Khayutin 1985). However, nestlings in the latter stages of development may freeze, cease begging, or crouch low in the nest upon hearing alarm calls given to warn of a predator near the nest (Ryden 1980; Kleindorfer et al. 1996; Davies et al. 2004; Platzen & Magrath 2004). If a predator persists in the vicinity of a nest and encounters young that are old enough to survive outside of the nest, such nestlings may fledge from the nest prematurely to avoid being preyed upon (Skutch 1976; Remes & Martin 2002). In at least one species, offspring have been reported to fledge from the nest after exposure to parental alarm calls (Suzuki 2011), indicating that simply hearing parental alarm calls may be enough to initiate fledging in some species. Comparative data indicate that the likelihood of premature fledging is strongly correlated with nest predation rate, which suggests that premature fledging is adaptive and undertaken by nestlings to maximize their likelihood of survival when confronted with a predator (Remes & Martin 2002). Such premature fledging is not

without costs, however, as leaving the nest early results in a reduced body mass at the time of fledging and is thought to reduce subsequent survival (Remes & Martin 2002). As such, premature fledging appears to occur only when nestlings are confronted with imminent danger from a predator. Taken together, available evidence indicates that parental alarm calls can communicate the presence of a predator near the nest and may lead older nest-bound young to undertake behaviors that reduce their risk of predation, including premature fledging from the nest.

Although it is clear that parental alarm calls can induce behavioral changes in offspring, little is known about whether parental alarm calls initiate a corticosterone response in older offspring that could serve to ready offspring for an interaction with a predator at the nest (Hollen & Radford 2009). Nevertheless, such a physiological response by nestlings to parental alarm calls might be expected because altricial nestlings typically are unable to defend themselves against predators, and premature fledging from the nest appears to be the only way by which nestlings can increase their survival prospects when confronted by a predator. To date, only a single study has tested this idea, and that study found that exposure to parental alarm calls did not initiate a corticosterone response in semi-altricial nestlings of the American kestrel (*Falco sparverius*; Dufty & Crandall 2005). That alarm calls did not lead to a corticosterone response in offspring may be because the cavity nests of the American kestrel are relatively safe against nest predators, unlike the open-cup nests of most passerines (Martin & Li 1992; Dufty & Crandall 2005), and safe nest sites may not exert selective pressure for offspring to initiate a corticosterone response that allows them to prematurely fledge the nest. Given its limited study, additional research on this topic is warranted, particularly for altricial species with high nest predation rates that are known to prematurely fledge from the nest when confronted by a predator.

In this study, we tested the alarm call-corticosterone hypothesis which states that exposure to parental alarm calls initiates a corticosterone response in physiologically mature altricial nestlings that readies them for a predator encounter at the nest. This hypothesis predicts that (1) exposure to parental alarm calls causes an elevation in the circulating corticosterone of a nestling and, in turn, (2) elevated corticosterone levels increase the likelihood that a nestling will fledge prematurely if confronted by a predator at the nest. Because the process of capture

and handling older nestlings causes nestlings to attempt to flee from the nest and elevates plasma corticosterone levels, manipulating corticosterone levels in older nestlings is extremely difficult under natural conditions. Therefore, this study focuses on testing the first component of this hypothesis to assess whether exposure to parental alarm led to an increase in circulating corticosterone levels in older nestlings.

For this study, we selected the white-crowned sparrow (*Zonotrichia leucophrys*, hereafter sparrow) because it experiences a high rate of nest predation, because older nestlings are known to prematurely fledge from the nest when disturbed, and because adult sparrows give alarm calls to communicate predation risk to nestlings when predators are near the nest (Hill & Lein 1985; Rivers, J. W., Martin, L. B., Liebl, A. L. & Betts, M. G., unpubl. data). If exposure to parental alarm calls in older sparrow nestlings causes an increase in circulating corticosterone, two conditions should hold. First, because altricial nestlings are unable to survive out of the nest during much of the nestling stage, a corticosterone response should only be mounted by individuals that are of an age where they can survive away from the nest. Second, because elevated levels of corticosterone has been shown to reduce growth in sparrows (Wada & Breuner 2008), only nestlings exposed to parental alarm calls should exhibit a corticosterone response, whereas nestlings without exposure should lack such a response.

It is worth noting that for the corticosterone response to promote premature fledging as an anti-predation response, some time is required for offspring to mount a corticosterone response after hearing parental alarm calls, as there is a short time lag between exposure to an environmental stressor and an increase in circulating corticosterone levels (Wingfield et al. 1998). Previous work on sparrow nestlings found that the corticosterone response is mounted 4–15 min after a stressor (Wada et al. 2007), suggesting that the amount of time between the onset of parental alarm calling and an attempted nest predation would need to be at least this long for offspring to initiate a corticosterone response after hearing adult alarm calls. Although we lack data on the full suite of nest predators in the community, our research group has found that the garter snake (*Thamnophis sirtalis*) and small mammals (e.g. Douglas squirrel [*Tamiasciurus douglasii*], *Tamias* spp.) are common predators of altricial Swainson's thrush (*Catharus ustulatus*) fledglings on our study sites (Rivers, J. W., Martin, L. B., Liebl, A. L. & Betts, M. G.,

unpubl. data) and we suspect they are important predators for sparrow offspring too. Given that even a single nestling can be a substantial meal for these small predators, such predators are likely to spend several minutes (or more) searching for a nest, particularly if they use parental alarm calling as a cue that a nest is nearby, and this period could provide an adequate time period for alarm calls to initiate a corticosterone response in sparrow offspring.

Methods

Study Species and Area

We conducted this work during the 2008 breeding season (May–July) on regenerating Douglas-Fir (*Pseudotsuga menziesii*) stands (ca. 7–12 yr old) that were located on private industrial forestlands in the Coast Range of western Oregon. The sparrow is a common nesting species in young forest stands, nesting in dense vegetation close to the ground and experiencing high predation rates that are typical of open-cup nesting passerines (see Results). Sparrows have a nestling period that lasts 8.5–10 d but are known to prematurely fledge from nests 1–2 d prior to the typical departure age when disturbed (Chilton et al. 1995; Rivers, J. W., pers. obs.). Adult sparrows have a variety of calls, with the 'pink' alarm call given by adults when predators are near the nest, including the red-tailed hawk (*Buteo jamaicensis*), Steller's jay (*Cyanocitta stelleri*), and humans (Hill & Lein 1985; Rivers, J. W., pers. obs.). The 'pink' alarm call differs markedly than the 'flee' alarm call that is given in response to predators that prey upon adult sparrows (e.g. *Accipiter* hawks): 'pink' alarm calls are short in duration (<0.1 s), cover a wide frequency range (3–7 kHz; see Hill & Lein 1985), can be given at a high rate (up to 1.5 calls per s; Rivers, J. W., unpubl. data), are often broadcast by individuals from an exposed perch, and may function as a mobbing call (Hill & Lein 1985). In contrast, 'flee' alarm calls are of constant frequency and are given sparingly as birds move rapidly into dense, protective cover (Rivers, J. W., pers. obs.). Thus, the 'pink' alarm call is given in response to predators that are threats to the nest but not to threats on adult sparrows, indicating this vocalization is used by adults to convey the risk of nest predation. Although the 'pink' alarm call could be used as a distraction display, parents typically do not use them in such a manner in our study area (Rivers, J. W., pers. obs.). It should be noted that older nestling sparrows often crouch low in nests and cease begging when exposed

to 'pink' alarm calls (Rivers, J. W., pers. obs.), suggesting this vocalization may serve to signal the presence of a predator near the nest to nest-bound young.

To find sparrow nests, we used systematic and behavioral approaches, and nests were monitored every 3–4 d until hatching after being located. For nests that were found after hatching, we aged young based on the descriptions in Banks (1959) in conjunction with our extensive experience with aging passerine nestlings. We conducted alarm call experiments on each nest at two ages: 3–4 d after hatching (hereafter young nestlings) and then again at 7–8 d after hatching (hereafter old nestlings). These comprised two distinct developmental periods of the nestling stage in sparrows, one where nestlings are unable to survive out of the nest (young nestlings) and one where nestlings are able to survive out of the nest if forced from the nest by a potential predator (old nestlings). The behavior of nestlings in each stage is markedly different, with young nestlings often begging when handled and showing no fear of humans, whereas older nestlings do not beg in the presence of humans and instead crouch low in the nest and remain immobile (Rivers, J. W., pers. obs.).

Field Procedures

Prior to conducting experiments, we randomly assigned each nest to one of the two treatments, balanced for nest initiation date to ensure equal representation of both treatment groups over the breeding season. A single experimenter (JWR) conducted all experiments on fair days without precipitation between 11:30 and 18:00 Pacific Daylight Time (PDT) to minimize any possible circadian variation in nestling corticosterone. Prior to conducting an experiment, he moved to a location ca. 50 m from the focal nest and remained hidden in a portable blind which allowed him to detect any 'pink' alarm calls that were given near the focal nest. Once a 30-min buffer period passed during which alarm calls were not given in the immediate vicinity of the nest (<25 m), he immediately proceeded with the experiment. Although it is unknown how long it may take corticosterone levels to return to baseline levels in sparrow nestlings after hearing 'pink' alarm calls, we selected a 30-min buffer period because previous work on European starling (*Sturnus vulgaris*) adults found that corticosterone levels returned to baseline levels 30 min after being released from restraint in a cloth bag (Rich & Romero 2005). On the few occasions where parents gave 'pink' alarm calls at the initial

placement of the blind, they ceased giving alarm calls within a short period (typically 5–10 min). At one nest, sparrow parents never ceased alarm calling at the placement of the blind despite multiple attempts on separate occasions, so no experimental procedures were conducted on this nest.

For nests in the control group, once the 30-min buffer period was met, the experimenter quickly, yet quietly, approached each nest, randomly selected two of the oldest nestlings for removal, placed them into a plastic container containing an artificial nest, and secured the top of the container, which had adequate ventilation holes for breathing. The experimenter then moved nestlings to a location ca. 50 m away from the nest where an initial blood sample was taken (hereafter the baseline sample). Baseline samples were collected within 4 min of the initial handling of nestlings or from the time the first 'pink' alarm call was given by parents during the approach to the nest, whichever came first. A 4-min cutoff was used for baseline samples because recent work with sparrow nestlings found that corticosterone levels were not elevated in the first 4 min after being handled (Wada et al. 2007). We took blood samples (ca. 75 μ l) from nestlings by pricking the alar vein with a sterile 27-gauge needle and collecting blood into heparinized capillary tubes. After bleeding, blood flow was staunched with cotton and blood samples were immediately placed on ice. At the time nestlings were bled, both nestlings were marked minimally with a non-toxic black felt marker for individual identification and then returned to the artificial nest which was placed in a secure location. Following the standard stress response protocol (Romero & Reed 2005), a second sample (hereafter the handling-induced sample) was taken approx. 60 min after the first blood sample. This second blood sample was taken to determine whether nestlings were physiologically capable of mounting a corticosterone response due to capture and handling by the experimenter, which served as a simulated predator encounter (Romero & Reed 2005). The second blood sample was taken from nestlings 60 min after the baseline sample because Wada et al. (2007) demonstrated that the corticosterone levels are elevated for at least 60 min after initial handling in sparrows when nestlings are old enough to mount a corticosterone response. We did not take blood at other sampling periods (e.g. 15 or 30 min after baseline) to limit the amount of blood taken from nestlings. After the handling-induced sample was taken, the experimenter measured the mass and tarsus length of each nestling and then returned them to their

nest, with all individuals being returned within 75 min of removal.

In experimental nests, we followed a protocol implemented by previous researchers studying the influence of parental alarm calls on offspring behavior whereby the experimenter stopped approx. 3 m from the nest to elicit alarm calls from parents (e.g. Kleindorfer et al. 1996). Upon reaching this location, the experimenter stood still, recorded parent alarm calls with a camcorder, and remained out of visual contact with the nestlings so that parental alarm calls were the only cues available to nestlings as to the presence of a predator near the nest. Once a 'pink' alarm call was given within 25 m of the nest, he noted the time and allowed parents to continue giving alarm calls during a 4-min period. We used this approach because it avoided potential complications that might arise during alarm call playbacks (e.g. artificially inflating natural alarm call rates by using alarm call playbacks; see Dufty & Crandall 2005) and it ensured that focal nestlings were exposed to alarm calls given by their parent(s) in the event that offspring responded to alarm calls of their parents differently than to those of neighboring adults. On the occasions where parents were away from the nest when the experimenter reached his position, he waited for their return and allowed them to give 'pink' alarm calls for 4 min to ensure that nestlings in all experimental nests experienced parental alarm calls for the same duration. In all cases, one or both parents gave 'pink' alarm calls to the experimenter's presence near the nest throughout the 4-min exposure period (see Results). After 4 min of alarm calling elapsed, we followed the same procedures used at control nests to remove nestlings from the nest and take blood samples. This approach allowed us to expose nestlings in the treatment group to a single stressor (i.e. 'pink' alarm calls) and ensured that any observed difference in mean levels of baseline corticosterone between control and experimental groups was because of the exposure of treatment nestlings to alarm calls. It should be noted that our study design was unable to accommodate an additional treatment group in which nestlings were exposed to control stimuli because the high predation rate greatly reduced the number of available nests. Given our results, however, such a treatment would not have provided any meaningful additional information. It should also be noted that when old nestlings were initially removed from the nest by the experimenter to take baseline blood samples, they typically attempted to escape and this behavior continued throughout the time

during which they were held for the second bleeding, suggesting that nestlings did clearly perceive the experimenter as a dangerous threat as has been assumed for other studies investigating the corticosterone response in birds (Wingfield et al. 1998).

Laboratory Procedures

Within 8 h of being drawn, we centrifuged blood in the laboratory at 4650 *g* for 10 min, removed the plasma, and then froze it at -20°C . At the completion of field work, we shipped samples to the University of South Florida on dry ice where laboratory analysis took place. We used a commercially available EIA kit (cat# 900-097; Assay Designs, Ann Arbor, MI) to measure corticosterone from plasma (Breuner et al. 2006). Briefly, 10% steroid displacement reagent (5 μl) was added to 5 μl of plasma; 5 min later, assay buffer (240 μl) was added to each sample, vortexed, and aliquoted in duplicate (100 μl per well) to assay plates. Standard curves (ranging from 200 000 to 32 pg/ml) were measured in duplicate on all plates. Samples were then incubated with conjugated corticosterone and antibody for 2 h at room temperature while being shaken. Wells were emptied and washed three times before pNpp substrate was added to all wells; plates were incubated 1 h at room temperature without shaking. Stop solution was then added and each plate was read at 405 nm (corrected at 590 nm). The detection limit for the assay is approx. 27 pg, and cross-reactivity is negligible (maximum with deoxycorticosterone at approx. 10%). Samples were randomly allocated to two plates and intra- and inter-plate variation was 11% and 8%, respectively.

Statistical Analysis

First, we used paired *t*-tests to determine whether physical characteristics (i.e. body mass and tarsus length) differed in nestlings in the control and experimental groups. Next, we analyzed corticosterone response data as a split-plot design with repeated measures separately for both of the ages at which nestlings were examined. Each of the two treatments was considered the whole-plot factor that was assigned to each nest, and the time of blood sampling (i.e. baseline or handling-induced) was considered the subplot factor assigned within each treatment. Split-plot ANOVA models were conducted using PROC MIXED in SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA) using the Kenward-Rogers method to calculate df. Type III *F*-tests of

hypotheses were used for all factors and interactions, significance levels for all tests set at $p < 0.05$. Unless otherwise noted, we report a mean and its respective 95% confidence interval (CI) for response variables.

Many studies examining the corticosterone response have involved small sample sizes, and virtually all have lacked an assessment of the ability of researchers to detect treatment differences (Cockrem et al. 2009). Therefore, we examined whether our study had the statistical power necessary to detect treatment effects of alarm calling on nestling corticosterone response by calculating the mean effect size as described by Steidl et al. (1997). The mean ($\pm 95\%$ CI) observed effect was calculated as the control value minus the mean treatment value using the 'diff' option in the PROC MIXED modeling function. With this approach, if the 95% CI for the observed effect overlaps with zero and excludes all effects considered biologically significant, the null hypothesis of no biologically significant effect can be 'accepted' (see Steidl et al. 1997 for details). It should be noted that estimating treatment effects and their associated confidence intervals is far more useful than conducting a retrospective power analysis on existing data; the flaws of the latter approach have been detailed elsewhere (Thomas 1997; Hoenig & Heisey 2001; Colegrave & Ruxton 2003; Nakagawa & Cuthill 2007). In this study, we focused on detecting a treatment effect in baseline samples taken from old nestlings, as a difference in corticosterone levels between control and treatment groups were predicted *a priori* for nestlings of this age. Specifically, if parental alarm calls had an effect on the corticosterone response, we expected that the minimum biological effect would be a doubling of the baseline corticosterone level in the treatment group relative to the control group for old nestlings. Given that recent work has shown that old sparrow nestlings of similar age have an increase in corticosterone owing to human handling that is $>4\times$ higher than baseline levels (Wada et al. 2007), our expectation of a doubling of corticosterone in response to alarm calls would therefore be considered a conservative estimate for detecting treatment effects. For all statistical tests, we only included the first nestling bled because of the strong potential for non-independence in the corticosterone response between siblings.

Results

Of 48 nests that were found and monitored until failure or fledgling, 19 nests fledged for an apparent nest success rate of $60\% \pm 7.1$ SE. Fourteen nests

were tested in the field (i.e. seven control nests and seven experimental nests), but these sample sizes were reduced during later sampling periods because of nest predation and logistical constraints. Despite these modest sample sizes, however, treatment effect sizes were large enough to detect biological differences between control and treatment nestlings, had they existed.

Nestlings in the two groups did not differ in their physical characteristics. For young nestlings, the mean mass of the first nestling bled in control nests (11.0 g [9.5, 12.5]) was similar to that of the first nestling bled in experimental nests (10.6 g [9.0, 12.2]; $t_{12} = 0.4$, $p = 0.684$), with a similar pattern found for tarsus length (control nests: 15.8 mm [14.7, 16.9], experimental nests: 15.6 mm [14.1, 17.0]; $t_{12} = 0.4$, $p = 0.735$). Likewise, for old nestlings, the mean mass of the first nestling bled in control nests (16.4 g [14.0, 18.8]) was similar to that of the first nestling bled in experimental nests (16.1 g [15.1, 17.2]; $t_8 = 0.3$, $p = 0.787$), with a similar pattern found for tarsus length (control nests: 22.1 mm [21.0, 23.1], experimental nests: 21.4 mm [20.6, 22.3]; $t_8 = 1.2$, $p = 0.251$).

Adults responded to the presence of the experimenter near the nest in all experimental trials by giving 'pink' alarm calls, with an average of 139 'pink' alarm calls per trial (range: 43–455 calls per trial). However, no association was found between the baseline corticosterone and the number of parental alarm calls to which offspring were exposed for either young (Pearson's $r = 0.494$, $p = 0.319$) or old nestlings (Pearson's $r = 0.318$, $p = 0.682$) in the treatment group. Mean total corticosterone of young nestlings did not differ between control and treatment groups ($F_{1,9,1} = 0.5$, $p = 0.492$) or between baseline and handling-induced samples ($F_{1,9,5} = 0.0$, $p = 0.862$) with no significant treatment \times time interaction ($F_{1,9,5} = 0.0$, $p = 0.950$; Fig. 1a). In contrast, mean total corticosterone of old nestlings differed significantly between baseline and handling-induced samples ($F_{1,8,0} = 6.0$, $p = 0.040$) but not between control and treatment groups ($F_{1,8,0} = 0.1$, $p = 0.780$) with no significant treatment \times time interaction ($F_{1,8,0} = 0.1$, $p = 0.723$; Fig. 1b). Our assessment of the statistical power to detect biologically significant effects found a mean observed effect size of -2.26 [-12.99 , 8.47] ng/ml that overlapped with zero but neither of the minimum biological effects (i.e. ± 16.12 ng/ml), which were estimated from the baseline total corticosterone of 8.06 ng/ml in control nests (Fig. 2). Thus, despite modest sample sizes, our data had the

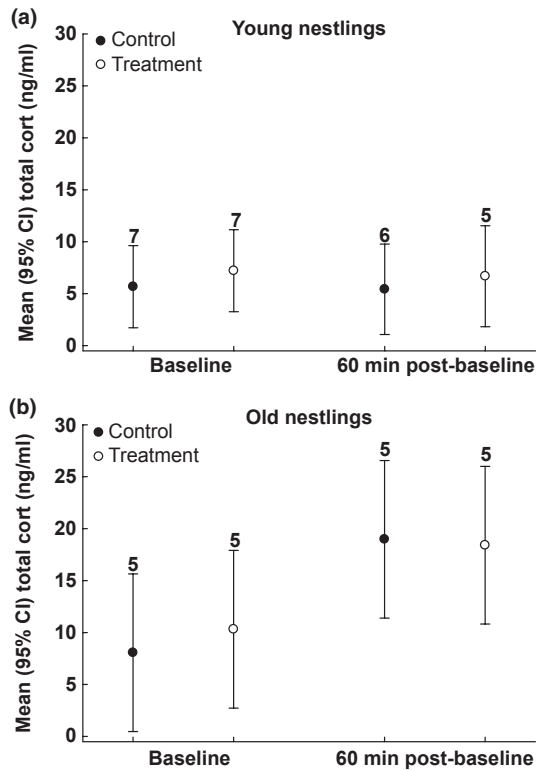


Fig. 1: Mean (95% CI) for total corticosterone (ng/ml) in circulating plasma of (a) young (i.e. 3–4 d after hatching) and (b) old sparrow nestlings (i.e. 7–8 d after hatching) taken at baseline and 60-min post-baseline periods.

power to detect biologically significant effects had they been present.

Discussion

As expected, young nestlings that were unable to survive outside of the nest did not elevate corticosterone levels after being exposed to parental alarm calls or in response to investigator handling. Contrary to our predictions, however, old nestlings that were exposed to parental alarm calls did not exhibit elevated corticosterone relative to old nestlings that were not exposed to alarm calls. This is noteworthy because old nestlings in both control and treatment groups regarded humans as a potential threat and attempted to fledge from the nest when handled, indicating they were able to recognize the danger posed by a potential predator and undertake appropriate behaviors. Moreover, old nestlings in both groups exhibited a significant increase in corticosterone after being handled, indicating they were capable of mounting a corticosterone response in conjunction with anti-predator behaviors. Although

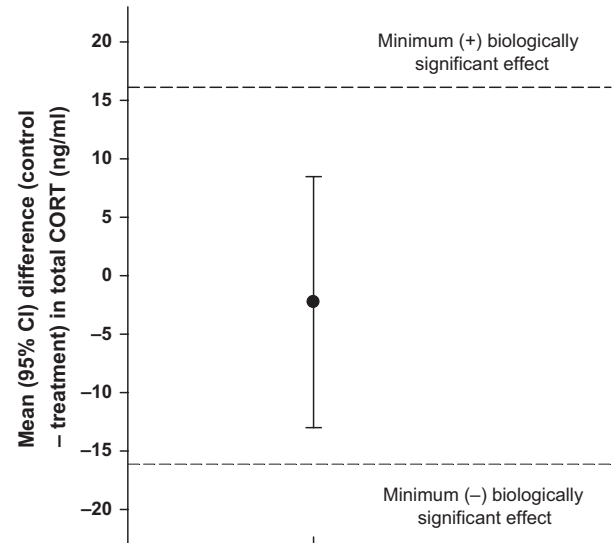


Fig. 2: Mean (95% CI) difference in total corticosterone (ng/ml) between old nestlings (i.e. 7–8 d after hatching) in control and treatment groups during the baseline period and *a priori* minimum biologically significant effects. Because the 95% CI for mean difference in total corticosterone did not overlap with either of the minimum biological effects, our study would have detected treatment effects had they been present. See text for additional details.

our sample sizes were modest, our effect size analysis indicated that our data had the power to detect biologically significant effects, had they been present. Put another way, conducting additional experiments would not have altered the conclusions made based on these data. Thus, the lack of a corticosterone response in old nestlings when exposed to parental alarm calls appears to be a real phenomenon that must be explained based on biological grounds.

Why did old nestlings lack a corticosterone response when exposed to parental alarm calls? One explanation for this finding is that parental alarm calls do not function as signals from parents to offspring about the threat of a predator but instead are used to recruit other birds to the nest area for predator mobbing (Curio 1978); indeed, ‘pink’ alarm calls did recruit local breeders to the area near the nest (Rivers, J. W., pers. obs.) as described by Hill & Lein (1985). However, even after other individuals arrived near the nest, parents continued to give this call throughout the 4-min calling period, which suggests it may have also been directed at nestlings. A second explanation is that parents gave alarm calls to teach offspring about predators, as predator recognition has been found to increase with age and experience in other species (e.g. Seyfarth et al. 1980; Francis et al. 1989; Hanson & Coss 2001; Hollen & Manser 2006). However, teaching offspring to

recognize predators requires that offspring associate an alarm call with the visual stimulus of a predator, which would appear unlikely in the case of the sparrow. Like many passerines with high nest predation rates, sparrows build well-hidden nests that are located in dense vegetation, which effectively restricts the view of nestlings to the immediate vicinity of the nest. Such a limited view would make it unlikely that offspring could make visual contact with a predator at a safe distance so as to associate it with parental alarm calls. In such well-hidden nests, predators would not be visually detected by offspring until immediately at the nest, so it may be that physical and visual contact are required to initiate anti-predation behavior and the accompanying corticosterone response. This may not be true of species nesting in more open areas; indeed, a recent study of the black-legged kittiwake (*Rissa tridactyla*) found that nestlings that were not handled, but had witnessed their nest-mate being removed by researchers, responded with an increase in corticosterone (Fridinger et al. 2007). Whether the sight of a predator alone can initiate a corticosterone response in sparrow nestlings remains an unknown yet interesting possibility that is worthy of additional study.

A final explanation to the pattern we observed is that the costs of mounting a corticosterone response when exposed to parental alarm calls might outweigh the benefits of such a response. Elevated corticosterone levels are known to be costly to developing young in terms of growth and development (Kitasky et al. 2003; Hayward & Wingfield 2004; Blas et al. 2006), including the sparrow (Wada & Breuner 2008), so it is possible that the costs of activating the corticosterone response after exposure to parental alarm calls outweighs the benefits of such a response. This could be important if the elevation of corticosterone occurs slowly and, in turn, is ineffective at promoting anti-predation behaviors of developing young. Such costs might lead to an even greater reduction in offspring fitness if alarm calls are given in error, or if alarm calls are given in response to predators that have a low probability of discovering the nest and confronting nestlings. Of note, old nestlings in this study did mount a corticosterone response after being handled, indicating they considered predation to be imminent when they were captured for bleeding by a human 'predator.' Such a response would appear to be an adaptive strategy that maximizes offspring fitness when a real danger is imminent, assuming that the cost of being captured is greater than the cost of reduced growth. A similar argument has been used to explain why

exposure to the odor of a predatory weasel (*Mustela erminea*) did not induce a corticosterone response in meadow voles (*Microtus pennsylvanicus*; Fletcher & Boonstra 2006) and reinforces the idea that direct evidence of a predator may be needed for mounting an acute corticosterone response.

Given that we did not find support for the corticosterone response as a mechanism facilitating premature fledging in sparrows, an alternative explanation implicates the 'fight or flight' response as playing a critical role in preparing nestlings to fledge prematurely from nests upon discovery by a predator, as older nestlings that fledge prematurely often attempt to leave the nest upon contact with researchers (Rivers, J. W., pers. obs.). This response differs from the corticosterone response in that it occurs within seconds after exposure to a stressor (as opposed to several minutes in the corticosterone response), lasts for a short time period and is initiated by the secretion of catecholamines from the sympathetic nervous system (Wingfield et al. 1998). The fight or flight response is notoriously difficult to study in free-living organisms under natural conditions, but additional study of other components of the vertebrate stress response (e.g. heart rate, immune function; see Romero 2004) will help distinguish which physiological responses, if any, are initiated when nestlings are exposed to parental alarm calls.

Although nestlings did not initiate a corticosterone response after exposure to alarm calls, they did initiate such a response upon being handled by a potential predator, and this might provide benefits to nestlings that survived such an encounter. Elevated corticosterone levels can lead to increased energy mobilization that shunts energy toward immediate survival needs and away from non-essential functions (Wingfield et al. 1998; Romero 2004). In turn, increased energy mobilization would facilitate increased locomotor activity and could allow nestlings to leave the general vicinity of the nest where the predator encounter occurred. Increased energy mobilization might be particularly important if parents cannot feed fledglings because of continued predator activity near the nest and offspring need to rely on body stores to sustain them until regular feeding can be reinstated, particularly because fledgling sparrows cannot feed themselves immediately after leaving the nest. Taken together, the initiation of the corticosterone response of old nestlings after contact with a human 'predator' appears to be adaptive, and likely functions in concert with the fight or flight response to ensure that nestlings escape from predators at the nest and are able to

sustain the physiological challenges that accompany early fledging.

Our work with sparrows revealed a pattern similar to a recent study of the American kestrel, in which nestlings lacked a corticosterone response after acute exposure to parental alarm calls (Dufty & Crandall 2005). That both sparrows and kestrels had similar responses yet differ in the safety of their nests suggests that the risk of nest predation does not influence whether parental alarm calls stimulate corticosterone production in offspring. Given this finding, it would be worthwhile to test the corticosterone response of a non-altricial species to parental alarm calls under natural conditions, as non-altricial species typically hatch with a functional hypothalamic–pituitary–adrenal axis (reviewed in Wada 2008; but see Adams et al. 2008) and have locomotory abilities that allow them to take evasive action against predators (Starck & Ricklefs 1998). Such a test would also offer insights into whether and how developmental mode influences offspring responses to parental alarm calls and would further our understanding of the costs of mounting a corticosterone response to natural stressors during development. Additional study of adults and older offspring than investigated here (e.g. dependent fledglings, independent juveniles) in other altricial species will be helpful in determining the extent to which older individuals mount a corticosterone response in conjunction with escape behaviors when exposed to alarm calls, and such studies will provide a better understanding of the physiological responses that accompany behaviors undertaken when under the threat of predation.

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