# Two seconds is all it takes: European starlings (Sturnus vulgaris) increase levels of circulating glucocorticoids after witnessing a brief raptor attack

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# Two seconds is all it takes: European starlings (*Sturnus vulgaris*) increase levels of circulating glucocorticoids after witnessing a brief raptor attack

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ABSTRACT

Researchers typically study "acute" activation of the hypothalamic–pituitary–adrenal (HPA) axis by measuring levels of circulating glucocorticoids in animals that have been exposed to a predator or a cue from a predator (e.g., odor), or have experienced a standardized capture-and-restraint protocol, all of which are many minutes in duration. However, exposure to predators in the "wild", either as the subject of an attack or as a witness to an attack, is generally much shorter as most depredation attempts upon free-living animals last <5 s. Yet, whether a stimulus lasting only seconds can activate the HPA axis is unknown. To determine if a stimulus of a few seconds triggers a glucocorticoid response, we measured levels of corticosterone (CORT; the primary avian glucocorticoid) in wild-caught European starlings (*Sturnus vulgaris*) after they witnessed a brief (<2–8 s) raptor attack upon a conspecific, a human "attack" (i.e., a researcher handling a conspecific), and an undisturbed control. Witnesses of a raptor attack responded with CORT levels comparable to that induced by a standardized capture-and-restraint protocol. Glucocorticoid levels of individuals following the control treatment were similar to baseline levels, and those that witnessed a human "attack" had intermediate levels. Our results demonstrate that witnessing a predator attack of very brief duration triggers a profound adrenocortical stress response. Given the considerable evidence of a role for glucocorticoids in learning and memory, such a response may affect how individuals learn to recognize and appropriately react to predators.



## Introduction

Levels of glucocorticoids (GCs; steroid hormones released by the adrenal cortex) at baseline serve critical metabolic functions, but increase following activation of the hypothalamic–pituitary–adrenal (HPA) axis in response to real or perceived stressors, both acute and chronic (Charmandari et al., 2005). Chronic exposure to GCs can deleteriously affect health and cognitive function in humans and animals (Cohen et al., 2007; McEwen and Sapolsky, 1995; McEwen and Wingfield, 2003; Roozendaal et al., 2009; Sheriff et al., 2009). Acute activation of the HPA axis facilitates physiological and behavioral changes that redirect energy away from processes not essential for immediate survival, such as reproduction, and toward processes and behaviors that may enhance survival, such as glucose mobilization, antipredator behaviors, and memory consolidation (reviewed by Sapolsky et al., 2000; Wingfield and Ramenofsky, 1999).

The conventional methods used to study acute activation of the HPA axis are often understood to be a proxy for a life-threatening encounter with a predator, even if not explicitly stated as such (Wingfield and Ramenofsky, 1999). However, the duration of experimental "acute" stressors used to date are typically longer, by orders of magnitude in most cases, than the duration of an acute predation attempt experienced by free-living animals. Methods that have been used include pursuit by a human for 15 min (Rödl et al., 2007), exposure to static predator mounts for 15 to 60 min (Cockrem and Silverin, 2002; Silverin, 1998), exposure to a tame individual of a predatory species that does not exhibit depredation behavior for 30 min (Canoine et al., 2002), or forced proximity to a live predator for 5 to 60 min (Canoine et al., 2002; Figueiredo et al., 2003; Manogue et al., 1975; McIntyre et al., 1999; Narayan et al., 2013; Park et al., 2008). Pakkala et al. (2013) present a notable exception with their use of a relatively brief predation stressor. They measured HPA axis responsiveness in rock pigeons (*Columba livia*) that were used as lure birds to attract and trap free-living raptors (see the Discussion section for further consideration of this study).

The most widely used stressor for assessing acute HPA axis responsiveness is the standardized capture-and-restraint protocol (Wingfield, 1994), in which an animal is captured and subsequently held in a cloth bag anywhere from 5 to 60 min while a series of blood samples are collected for later measure of GC (Astheimer et al., 1995; Small and Schoech, 2015; Wingfield et al., 1992). This method has been widely used to study the acute stress response of vertebrates

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(Cockrem, 2013), and restrained animals are thought to perceive the capture-and-restraint as a life-threatening encounter with a predator (Wingfield and Ramenofsky, 1999), which is arguably the ultimate acute stressor.

In contrast, most depredation attempts expose prey to a life threatening interaction lasting from mere seconds to just a few minutes. For example, most felids stealthily approach unsuspecting prey and quickly pounce and strike their target, typically without need of a chase (Fitzgerald and Turner, 2014; Leyhausen, 1979). Likewise, most raptors ambush and pursue potential prey for <5 s (Cresswell, 1996; Rudebeck, 1951, 1950). Peregrine falcons (*Falco peregrinus*) and cheetahs (*Acinonyx jubatus*) pursue prey for hundreds of meters, but rarely for more than 30 s (Cresswell, 1996; Schaller, 1968). Even pursuits of larger game by cooperative hunters typically last <5 min (e.g., Boesch, 1994; Creel and Creel, 1995; Mech, 1981; but see Ford et al., 2005). Animals commonly escape brief encounters with predators (reviewed by Vermeij, 1982), and thus live long enough to experience the presumed resulting GC response. Moreover, among social animals, witnessing brief predator attacks upon other individuals is likely a common occurrence. Indeed, individuals of many species learn the dangers of predators by observing interactions between predators and other animals (reviewed by Avarguès-Weber et al., 2013; Griffin, 2004). Although witnessing a predator attack often elicits emotional reactions and strong antipredator behavior in birds (Griffin, 2004; White et al., 2005), the nature of the adrenocortical stress response to any stimulus lasting only seconds is unknown. In fact, one might question whether such short-lived exposures produce a pronounced or even measureable GC response. Conventional methods are a valuable first step, but, given their unnaturally long duration and often contrived interactions, how well do they simulate the physiological stress response experienced by free-living animals in reaction to natural encounters with predators? In an attempt to address this question, we examined GC levels of individuals following extremely short-duration exposure to a predator.

We hypothesized that a stimulus lasting only seconds is a stressful event capable of activating the HPA axis, and thus predicted that observing a brief duration predator attack leads to activation of the HPA axis and ultimately, a GC response. We tested this prediction by measuring circulating levels of corticosterone (CORT; the primary GC in birds, rodents, reptiles, and amphibians) in wild-caught European starlings (*Sturnus vulgaris*, a social passerine) after they had witnessed both raptor and human attacks upon a conspecific. Though our protocol does not allow us to distinguish whether the observed CORT responses were due to witnessing an attack per se or observation of the attacker, we use the term "witness" throughout.

## Methods

### *Study species and site*

We obtained 24 free-living European starlings in Gorham, Maine and transported them to Block Island, Rhode Island (41°10′20″N, 71°33′27″W) in mid-September 2012. The Institutional Animal Care and Use Committee of the University of Memphis approved all protocols. Study subjects were a mix of adult and hatch year birds of both sexes, as determined by morphometrics, plumage, and iris color (Pyle et al., 1997). Of the 18 individuals used in the experiment (see below), 15 were adults. Starlings were divided into two equal groups and held in two, free-flight aviaries measuring 1.0 m × 2.5 m × 2.0 m for seven days. In each aviary we provided multiple perches and three locations with ad libitum food and open water for drinking and bathing. To allow ready access to individuals during the experimental period (23 Sep to 8 Oct), we randomly selected and paired 18 individuals into nine cages (36 cm × 43 cm × 60 cm). We housed starlings as pairs because isolation increases baseline CORT in this highly social species (Apfelbeck and Raess, 2008). We provided fresh food (a mixture of Kaytee Exact Softbill pellets, Eukanuba Maintenance Small Bite Dog Nutrition, hard-boiled egg, and layer mash chicken feed) and water ad libitum twice daily. We cleaned aviaries and cages daily and maintained starlings under natural photoperiod and ambient temperatures, but protected them from rain and wind.

### *Blood sample collection*

Circulating CORT concentrations begin to increase within 3 min of the onset of an acute stressor in passerines (Romero and Reed, 2005; Romero and Romero, 2002); therefore, with the exception of the capture-and-restraint stress-induced samples, we collected all samples within 3 min of the initial disturbance associated with blood sampling. To ensure CORT levels had begun to rise, but were unlikely to have exceeded peak levels (based on previous capture-and-restraint experiments conducted on starlings; Rich and Romero, 2005; Romero and Remage-Healey, 2000), we collected stress-induced blood samples approximately 11 min after the initiation of a stressor (i.e., capture-and-restraint or attack treatments; see below). Samples consisted of ≤120 μL of blood collected in heparinized microhematocrit tubes following puncture of the brachial vein with a 26 gauge needle. For a given individual, we collected no more than 240 μL of blood in any 24 h period or 720 μL over the entire study period, which are approximately half of the permissible volumes (Fair et al., 2010; Owen, 2011). Samples were held on ice for 1–3 h, after which plasma was separated by centrifugation, drawn off, and stored at −20 °C until analysis. Basal and stress-induced circulating CORT levels are highest during the non-active period (i.e., night), and transition to lower levels just before the onset of the active period (i.e., day) in diurnal birds (Breuner et al., 1999; Tarlow et al., 2003; Westerhof et al., 1994), including starlings (Romero and Remage-Healey, 2000). To control for circadian variation in CORT levels, we collected blood samples during the starling active period (at least 1.5 h after sunrise and no later than 2.5 h before sunset).

### *Standardized capture-and-restraint stress*

Four days before the initiation of predator attack trials, we collected blood samples from all individuals using a standardized capture-and-restraint stress protocol (modified from Wingfield et al., 1992). To ensure that all subjects were bled within 3 min (mean ± SD = 2.1 ± 0.7 min; range = 0.9–2.9 min) of initial disturbance, we maintained starlings in multiple, isolated locations. We then placed birds in a cloth bag in the shade until we collected a second blood sample approximately 11 min after initial disturbance (mean ± SD = 11.0 ± 0.9 min; range = 9.9–12.7 min).

### *Experimental treatments*

Eighteen starlings witnessed three different treatments on a focal lure starling: (*i*) a raptor attack (R); (*ii*) a human "attack," during which a researcher approached and handled the lure (H); and (*iii*) an unmolested control (C) (see below for details for each treatment). Witnesses were randomly assigned to one of 18 unique sequences of four exposures to the three treatments (e.g., RRHC, HCCR, CRHH); one treatment was repeated in each sequence for statistical considerations (see below). Starlings experienced no more than one treatment in a 24 h period (median = 48 h between treatments; range = 24–126 h). For each exposure event, we randomly assigned a starling to one of four observation cages (21 cm × 21 cm × 21 cm) atop a 1 m pole in a semicircular arrangement 4 m from the lure starling (Fig. 1). We positioned witness cages to allow direct visual observation of the lure starling while preventing visual observation of other witnesses and the blind, from which we controlled the lure (Fig. 1). Cages contained a perch and fresh food and water. All treatments occurred between 75 and 175 min after witnesses were placed in their observation cages to ensure that CORT levels measured during treatments had likely returned to pre-handling levels (Rich and Romero, 2005). We scheduled

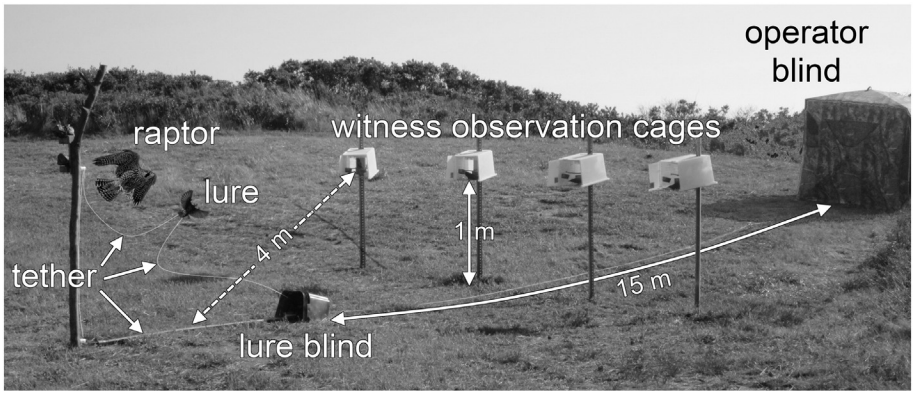


**Fig. 1.** Experimental set-up to elicit attacks from free-living raptors upon a European starling lure in view of four witness starlings arranged in a semicircle; all witness observation cages are equidistant from the lure.

the human and control treatments to approximate the timing of raptor attacks, which were largely out of our control (H = 101.4 ± 24.2 min, C = 104.5 ± 25.0, and R = 106.6 ± 25.0 min; all times reflect mean [±SD] time that had passed after witnesses were placed in their observation cages).

*Raptor attacks*

To elicit raptor attacks, we used methods commonly employed at raptor banding stations (Hull and Bloom, 2001). We tethered a lure starling, wearing a protective leather harness, to a Dacron® line. The line allowed manipulation of the lure from a blind positioned approximately 15 m from the lure and behind the witness cages (Fig. 1). The researcher in the blind periodically pulled on the line to lift the lure starling into the air, causing it to flap and, in turn, attract the attention of nearby raptors that were spotted from inside the blind, or were spotted by another biologist from approximately 50 m behind the blind and communicated to the blind via walkie-talkie. We used two dedicated lure starlings for the duration of the experiment; both experienced four attacks by raptors, but neither sustained visible injuries from these encounters. Three raptor species attacked the lures: peregrine falcon ($n = 6$), merlin (*Falco columbarius*; $n = 1$), and Cooper's hawk (*Accipiter cooperii*; $n = 1$). Peregrine falcons attacked the lure starling from on high in a single stoop and did not alight. In contrast, the merlin and Cooper's hawk approached the lure, flying much closer to the ground and concluded their attacks by landing on top of the lure. In all instances raptors made direct contact with the lure, and at the moment of contact the researcher in the blind used the tether to pull the lure into a small, camouflaged blind out of sight of the witnesses (Fig. 1). This caused the attacking raptor to release the lure and fly away. The duration of the approach, attack, and departure that was visible to witness starlings was <2 s for five peregrine falcons and <8 s for the remaining peregrine falcon attack, as well as those of the merlin and Cooper's hawk. After the raptor attacks, witness starlings remained undisturbed in their cages for approximately 11 min (mean ± SD = 11.0 ± 0.7 min; range = 10.0–12.0 min) until biologists collected blood samples within 3 min (mean ± SD = 1.7 ± 0.7 min; range = 0.8–2.8 min) of first human disturbance.

*Human attack*

As in the raptor attack treatment, the tethered lure starling was periodically pulled into the air preceding the human attacks. At the designated time, a biologist approached and grabbed the lure starling for <2 s, after which the researcher in the blind remotely pulled the lure starling into the camouflaged blind out of sight of the witness birds, and the "attacking" biologist quickly retreated. The "attacking" biologist wore the same wide brimmed hat, oversized sunglasses, and blue field clothes for each trial; besides the human attack, at no time did a researcher wear blue clothes, hats, or sunglasses during the study. After the human attacks, witness starlings remained undisturbed in their cages for approximately 11 min (mean ± SD = 10.8 ± 0.7 min; range = 9.9–12.0 min) until biologists collected blood samples within 3 min (mean ± SD = 1.6 ± 0.7 min; range 0.8–2.9 min) of the first human disturbance associated with blood collection.

*Controls*

As in the attack treatments, the tethered lure starling was remotely pulled into the air periodically preceding control sample collection. At the designated time, the researcher in the blind remotely pulled the lure bird into the camouflaged blind. Witness starlings remained undisturbed in their cages for approximately 11 min (mean ± SD = 10.9 ± 0.7 min; range = 10.0–12.0 min) until biologists collected blood samples within 3 min (mean ± SD = 1.8 ± 0.7 min; range 0.8–2.9 min) of first human disturbance.

*Plasma CORT analysis*

We used an enzyme-linked immunoassay (Cayman Chemical 500655; detection limit of 30 pg/ml) to quantify total plasma CORT, which we argue is the most appropriate and interpretable measure of CORT (Schoech et al., 2013). Prior to CORT assay, we diluted plasma samples in assay buffer (1:21 for baseline and control samples and 1:101 for stress-induced samples) to ensure values fell on the linear part of the standard curve (see Small and Schoech, 2015 for details). Samples were assayed in duplicate over three plates with intra-assay CVs of 3.5–3.7%. We used a known amount of standard CORT to prepare standard curves and internal controls. Inter-assay CV was 4.7%.

*Statistical analysis*

We used an 18-sequence, 4-period, 3-treatment crossover design, which allowed for the estimation of first-order carryover effects (i.e., residual effects due to the type of immediately preceding treatment). We did not estimate higher-order effects (i.e., the combined residual effects due to two or more preceding treatments), as these effects are not routinely considered and would have required an overly complicated design and unrealistic sample size (Williams, 1949). Additionally, a washout period (≥24 h between treatments) reduced the possibility of carryover effects in CORT measurements. Our design also allowed for the estimation of interactions between carryover and direct effects by considering "mixed" and "self" carryover effects (Kunert and Stufken, 2002). In each of the 18 unique treatment sequences, one treatment was repeated in two consecutive periods. That is, each treatment preceded every other treatment ("mixed"), as well as itself ("self"), the

same number of times. An estimation of all possible interactions between carryover and direct effects, as put forth by Sen and Mukerjee (1987), would require a model with too many parameters to be useful, but the use of mixed and self carryover effects offers an optimal compromise (Kunert and Stufken, 2002). This design further guaranteed that carryover effects were not confounded with treatment effects. Because period effects are common in crossover designs (e.g., acclimation to stressors), we designed sequences to guarantee that period effects were not confounded with treatment effects (i.e., each treatment occurred the same number of times in each of the four periods). We randomly assigned individuals to sequences.

We evaluated the effects of attack treatment on CORT levels using a linear mixed effects model in R (version 3.2.1; R Core Team 2015) with the "lme4" package (Bates et al., 2015). In addition to treatment, period, first-order carryover, and the type of first-order carryover effect (i.e., "self" or "mixed"), we allowed for a possible interaction between the type of carryover and first-order carryover effects. We also included four covariates in the analysis: the sex of the individual, the presence of active body or flight feather molt (Romero and Remage-Healey, 2000; Romero et al., 1998), time spent in the observation cage prior to the treatment, and witness baseline CORT level measured prior to the experiment. We did not evaluate the effect of age since most individuals were adults.

We estimated variances separately for each treatment to accommodate variance heterogeneity among the three treatments (Pinheiro and Bates, 2000). We included a random intercept for individuals, but excluded the random effect for the 26 attack and control events required to complete the experiment because there was no variability attributable to these events beyond residual variation. We report tests of fixed effects using the Kenward–Roger adjustment (Kenward and Roger, 1997) from the "pbkrtest" package in R (Halekoh and Højsgaard, 2014). We report probabilities from Tukey's adjusted pairwise comparisons, as well as effect size estimates (Cohen's *d* adjusted for matched measurements; Dunlop et al., 1996) between treatments.

To evaluate the similarity of CORT levels following exposure to a live predator attack upon a conspecific to those obtained in response to the capture-and-restraint protocol, we used two one-sided tests (TOST; Schuirmann, 1981; Westerlake, 1981) from the "equivalence" package in R (Robinson, 2014). Additionally, we used TOST to evaluate the similarity of CORT levels following exposure to a control treatment to baseline levels obtained during the capture-and-restraint protocol. We considered mean CORT levels of witnesses to be equivalent if they differed by less than the standard deviation of the relevant capture-and-restraint CORT measurements (i.e., 3.6 ng/ml for the comparison of experimental control vs. baseline CORT levels and 11.0 ng/ml for the comparison of experimental raptor attack vs. capture-and-restraint stress-induced CORT levels).

All data and R code necessary to replicate these analyses are available at https://github.com/adamdsmith/Starling_CORT.

## Results

Levels of CORT varied markedly and distinctly among all experimental treatments ($F_{2,\ 27.1} = 85.5$, $P < 0.001$; Fig. 2). Specifically, CORT levels of individuals that witnessed a raptor attack were markedly higher than their CORT levels when in the control treatment (least squares mean difference ± SE = 27.4 ± 3.0 ng/ml; $t_{22.8} = 9.1$, $P < 0.001$; Cohen's $d = 2.6$) or after witnessing a human attack (least squares mean difference ± SE = 13.3 ± 3.2 ng/ml; $t_{29.0} = 4.1$, $P < 0.001$; Cohen's $d = 1.4$). CORT levels of starlings that witnessed a human attack were also considerably higher that those that resulted from the control treatment (least squares mean difference ± SE = 14.2 ± 1.4 ng/ml; $t_{22.3} = 10.2$, $P < 0.001$; Cohen's $d = 2.0$). TOST indicated equivalence between the CORT levels of starlings after a raptor attack and pre-experimental capture-and-restraint ($P = 0.006$), as well as between experimental controls and pre-experimental baseline levels ($P = 0.002$).

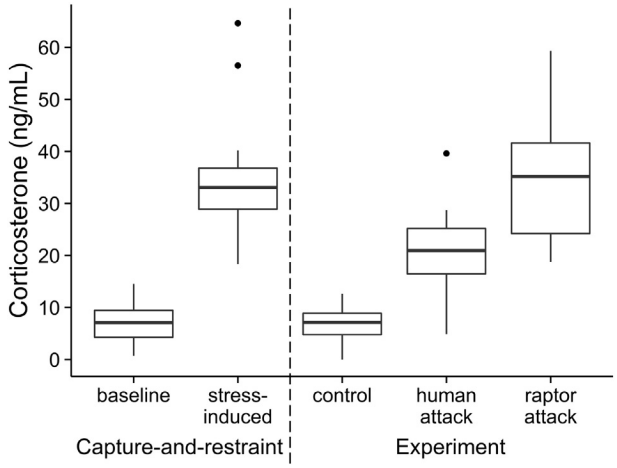


**Fig. 2.** Total plasma corticosterone measured from 18 starlings during capture-and-restraint and three experimental treatments. All samples were collected within 3 min of initial human contact. See text for a description of the capture-and-restraint and experimental treatments. Results represented as Tukey boxplots.

Because there was little evidence of an interaction between first-order carryover effects and the general type of carryover effect ($F_{2,\ 39.2} = 1.0$, $P = 0.49$), we excluded this interaction from the final model, and thus included only the additive effects of first-order carryover (i.e., did the type of treatment in the previous period influence CORT levels in the current treatment?) and general carryover type (i.e., "self" or "mixed"). The distinction between "self" and "mixed" carryover effects in CORT levels was inconsequential ($F_{1,\ 31.7} = 1.6$, $P = 0.21$), as was the influence of the preceding treatment on the current treatment (i.e., first-order carryover effects; $F_{2,\ 31.0} = 1.8$, $P = 0.18$). Starling CORT levels did not vary with the sequence of the treatments (i.e., period effects; $F_{3,\ 22.3} = 1.7$, $P = 0.19$). We found no evidence that baseline CORT level ($F_{1,\ 13.3} = 1.4$, $P = 0.25$), molt status ($F_{1,\ 14.0} = 0.0$, $P = 0.96$), or sex ($F_{1,\ 14.3} = 1.5$, $P = 0.24$) influenced the stress response of witness starlings. Witnesses that waited longer in the observation cage prior to treatment exhibited slightly increased levels of CORT ($F_{1,\ 23.3} = 4.3$, $P = 0.05$). Specifically, CORT levels varied approximately 5 ng/ml over the full range of times spent in observation cages, or roughly 17% of the observed effect of witnessing a raptor attack. This minimal effect suggests that the HPA axes of individuals with shorter wait times between transfer to observation cages and witnessing raptor attacks were still experiencing some degree of negative feedback.

The fixed effects in our final linear mixed model (predominantly treatment effects) explained roughly 59% of the variation in CORT measurements (marginal $R^2 = 0.59$; Nakagawa and Schielzeth, 2013). We do not report the conditional $R^2$ values (i.e., proportion of variation explained by fixed and random effects) given the slightly contrived random effects structure necessary to fit the heterogeneous variance model.

## Discussion

Our study demonstrated that starlings that witnessed (*i*) a fleetingly brief (<2–8 s) raptor attack upon a conspecific exhibited a pronounced elevation in levels of CORT comparable to that induced by a standard capture-and-restraint protocol, and (*ii*) an equally brief human attack exhibited increased levels of circulating CORT, but to a lesser degree than those induced by a raptor attack or handling stress. To the best of our knowledge, we are the first to demonstrate that such an exceptionally brief stimulus (<2–8 s) is sufficient to induce GC release.

Although starlings exhibited elevated levels of circulating CORT after witnessing a raptor attack, we cannot determine which specific stimulus or combination of stimuli are responsible. For instance, starlings

may have exhibited similar levels of CORT in response to a 2–8 s presentation of a relatively static, non-attacking raptor. Indeed, presenting a predator or predator model for extended periods of time (e.g., 15–30 min) is sufficient to activate the HPA axis (e.g., Canoine et al., 2002, Cockrem and Silverin, 2002), but exposure of shorter durations have not been tested. Additionally, lure starlings vocalized very briefly during both raptor and human attacks, but ceased as soon as the attack ended. Conceivably, short alarm calls from the attacked conspecific could have influenced levels of circulating CORT in witnesses. Alarm calls can induce profound physiological and behavioral effects in individuals that receive the calls (reviewed by Hollén and Radford, 2009), but the endocrine response of an animal to hearing an alarm call remains unknown. Still, other elements may have caused the elevated levels of CORT in witness starlings, such as non-vocal behaviors of the lure starling, close proximity to a fast moving object, the inability to escape after seeing a raptor, and the actual attack itself. Though we cannot determine which specific stimulus caused elevated levels of CORT in witness starlings, we suspect a combination of elements is responsible.

We are aware of only one other study that has evaluated HPA axis activation in response to a relatively brief encounter with a predator. Pakkala et al. (2013) documented the stress response of rock pigeons used as lures to trap free-ranging raptors. The authors report a nearly statistically significant increase of CORT levels in pigeons attacked by raptors relative to controls ($P = 0.06$; see Fig. 1 in Pakkala et al., 2013). However, the elevated CORT levels cannot be solely attributed to raptor attacks because the attacked birds experienced additional non-predator stimuli not experienced by controls. Specifically, 11 of the 18 attacked individuals were captured in a spring-loaded trap along with the raptor and experienced close proximity to at least one human and the trapped raptor as it was extricated from the trap. The authors do not indicate the duration of the stressor (i.e., raptor attack, trapping, and removal), but, based on our experience trapping raptors, the entire stimulus likely lasted 60 s or more.

In addition to predator exposure and capture-and-restraint, biologists have studied the GC response to a variety of "acute" stressors, including social isolation (Hennessy, 1997), novel environments (Hennessy and Levine, 1978; Muir and Pfister, 1986), and agonistic encounters with conspecifics (Huhman et al., 1990; Louch and Higginbotham, 1967; Øverli et al., 1999). Like predator cues and capture-and-restraint, the duration of stressful stimuli in studies such as these typically range from minutes to hours. However, methods of notable exception include 15 s of human handling (Armario et al., 1986), 30 s of repeated electric foot shocks (Friedman et al., 1967), and 30 s of placement on a hot plate (Galina et al., 1983), all of which increased circulating levels of plasma CORT in rats (*Rattus norvegicus*). Nonetheless, to the best of our knowledge, the <2–8 s raptor attacks in this study are the briefest stimulus shown to elicit a GC response in any animal, target or spectator.

Why did witnesses experience a reduced CORT response to the human "attack"? Starlings may regard humans as less dangerous than natural avian predators because of repeated non-antagonistic contact with humans leading to habituation (Rankin et al., 2009). Additionally, alarm calls produced by lure starlings during raptor and human attacks conceivably conveyed differential information to witnesses regarding the severity of the threat, thus influencing witness perceptions of danger and in turn their CORT responses.

The standardized capture-and-restraint protocol (Wingfield, 1994), used widely across vertebrate taxa to quantify the stress-induced adrenocortical response (Cockrem, 2013), produced an HPA axis response comparable to that induced by witnessing a live predator attack. However, because our capture-and-restraint and experimental protocols contained only a single sampling point, the two methods may have elicited fundamentally different CORT responses in terms of the maximal levels attained, time until peak CORT levels, or the duration of the response. An assessment of CORT concentrations at multiple time points following the predator attack stimulus would reveal the nature of the CORT response; however, this will require a between-subjects approach to avoid the confounding influence of repeated human handling and blood collection on the CORT response.

Increased levels of circulating GCs can enhance the consolidation of learned information into long-term memories (reviewed by McGaugh, 2000; Rodrigues et al., 2009; Schwabe et al., 2011). Many animal species rely on learned information to respond appropriately to predators (review by Griffin, 2004), and thus HPA axis sensitivity to witnessing a brief predator attack may be adaptive, particularly among social species. That is, an animal that experiences a CORT response immediately after witnessing a predator attack upon a conspecific may better remember that type of predator and employ more appropriate antipredator behaviors in future encounters, thus enhancing survival. Whether GC release during brief encounters with a predator conveys such beneficial effects to an individual's memory and survival remains untested, but is a worthwhile avenue of investigation with possible real-world applications, considering that many conservation programs expose naïve, captive-reared individuals of endangered species to predators prior to release in an attempt to increase their post-release survival (Griffin, 2004; McLean et al., 1999; Van Heezik et al., 1999; White et al., 2005).

## Conclusion

Our data demonstrate that witnessing a brief (<2–8 s) depredation attempt upon a conspecific activates the HPA axis, culminating in CORT release. However, the exact stimulus or stimuli responsible for the observed difference in CORT levels among treatments are unknown. The effect of this very brief and ecologically relevant stressor upon GC secretion was comparable to that induced by capture-and-restraint. We speculate that the observed HPA axis sensitivity in response to such a brief stimulus may be adaptive, given the enhancing effects of GCs upon memory consolidation, and this might be especially so for social species. Future studies should consider the temporal nature of GC secretion in response to witnessing a depredation attempt as well as other influences (e.g., conspecific vs. heterospecific targets and visual, auditory, and olfactory stimuli) on the magnitude of the response across the spectrum of sociality.

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