© 2017. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2017) 220, 322-327 doi:10.1242/jeb.144378

RESEARCH ARTICLE

Corticosterone rapidly suppresses innate immune activity in the house sparrow (*Passer domesticus*)

Sisi Gao*, Clarissa Sanchez and Pierre J. Deviche

ABSTRACT

Stress-induced effects on innate immune activity in wild birds have been difficult to predict. These difficulties may arise from the frequent assumptions that (1) the stress response influences different components of the immune response similarly, (2) stress-induced effects do not change over the course of the stress response and (3) glucocorticoids are the primary regulators of stress-induced changes of immune activity. We tested the first two assumptions by measuring three components of innate immunity at two times during the stress response in captive adult male house sparrows, *Passer domesticus*. Acute stress resulting from handling and restraint suppressed plasma lytic and microbicidal activity within 10 min and reduced plasma agglutination ability within 120 min. We tested the third assumption by measuring stress-induced effects in sparrows that were pharmacologically adrenalectomized by mitotane administration. Confirming the effectiveness of this treatment, mitotane-treated birds had lower pre-stress plasma CORT than control birds and showed no increase in plasma CORT during acute stress. The innate immune activity of mitotane-treated birds did not decrease during the stress response, but the pre-stress immune activity of these birds did not differ from that of vehicle-treated birds. These results suggest that elevated plasma CORT during stress is primarily responsible for mediating stress-induced suppression of innate immune activity.

KEY WORDS: Stress-induced immunosuppression, Innate immunity, Stress, Mitotane, Glucocorticoid, Mineralocorticoid receptor

INTRODUCTION

In vertebrates, the stress response adjusts physiology and behavior (e.g. inhibits reproduction and mobilizes glucose) to improve chances of surviving a stressor. Stress-related adjustments are orchestrated by a transient increase in catecholamine and glucocorticoid secretion (Sapolsky et al., 2000) and include a change in activity of the innate immune system, which serves as the most immediate line of defense against invading pathogens (Martin et al., 2008; Hasselquist and Nilsson, 2012). Stress-induced effects on innate immune activity have been investigated in free-living animals, but the direction of these effects remains difficult to predict. Stress-induced immunosuppression may occur because the animal lacks the resources that are necessary to sustain the activation and maintenance of multiple physiological systems (Martin et al., 2008; Moore and Hopkins, 2009; Nebel et al., 2012; Evans et al., 2015). Alternatively, animals may benefit from stress-induced immunoenhancement because exposure to stressors (e.g. predators and infectious agents) may increase the probability of injuries and infections (Dhabhar, 2009; Martin, 2009). Finally, stress-induced effects on immune activity may change as a function of the duration of exposure to the stressor, such that immunoenhancement occurs initially but immunosuppression occurs during a prolonged stress response (Martin, 2009).

Empirical evidence of stress-induced effects on the immune system has been equally conflicting. Many studies on this topic have used free-ranging birds as models because the diversity of habitats used by birds and of life history characteristics among species may help identify broad patterns of stress-induced effects on the immune response (Hasselquist, 2007). Several avian species exhibit stress-induced immunosuppression such as reduced microbicidal (Matson et al., 2006; Merrill et al., 2012) and lysozyme activity (Zylberberg, 2015), decreased natural antibody and complement-mediated activity (Davies et al., 2016), and reduced cutaneous immune activity (Martin et al., 2005; Cyr et al., 2007). Stress, however, has also been observed to enhance acute phase protein activity in the Galápagos flycatcher (*Myiarchus magnirostris*; Zylberberg, 2015) and phagocytic activity in clay-colored thrushes (*Turdus grayi*; Millet et al., 2007). These apparently inconsistent findings may result from most studies using one measure of immunity and/or sampling at one point during the stress response. In red knots (*Calidris canutus*), for example, the activation of the stress response enhances phagocytic ability and reduces plasma leukocyte concentration at different times during the stress response (Buehler et al., 2008). In such cases, stress-induced effects could not be properly characterized based on one measure of immunity and/or sampling at one time during the stress response.

Elevated plasma glucocorticoids inhibit immune activity in laboratory rodents and in clinical studies (Franchimont, 2004). These effects are mediated by the activation of glucocorticoid receptors (GR) and may involve both genomic and non-genomic mechanisms (Stahn and Buttgereit, 2008). In free-ranging birds, by contrast, it is unclear whether corticosterone (CORT), the primary avian glucocorticoid (Schmidt et al., 2010), plays a role in the regulation of stress-induced effects on immunity and whether plasma CORT can directly alter immune activity (Davies et al., 2016). Most studies on this subject have been correlative and do not, therefore, establish a causal relationship between changes in plasma CORT and immune activity (Lindström et al., 2005; Matson et al., 2006; Cyr et al., 2007; Buehler et al., 2008; Zylberberg, 2015). Furthermore, demonstrating that elevated plasma glucocorticoids are the primary regulators of stress-induced changes in immune activity is complicated by the fact that the environment can also influence the development and the function of the immune system (Hasselquist and Nilsson, 2009). In addition, plasma catecholamines, which rapidly increase during stress, may also reduce immune activity (Brown-Borg et al., 1991; Denno et al., 1994; Sapolsky et al., 2000; Martin, 2009). Most

School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA.

*Author for correspondence (sisi.gao@asu.edu)

S.G., 0000-0003-4018-2171

Received 6 June 2016; Accepted 28 October 2016

List of abbreviations

BKA	bacterial killing assay
BL	baseline
CFU	colony-forming units
CORT	corticosterone
GR	glucocorticoid receptor
MR	mineralocorticoid receptor
PBS	phosphate buffered solution

studies have used CORT administration or supplementation in food or drinking water to try and establish a causal relationship between this hormone and immune activity in free-living birds. However, this approach can result in supraphysiological (Loiseau et al., 2008) or chronically elevated plasma glucocorticoids (Martin et al., 2005; Bourgeon and Raclot, 2006) and so does not necessarily demonstrate a role for transiently elevated plasma glucocorticoids as naturally experienced during acute stress.

Here we tested the hypothesis that activation of the stress response changes innate immune activity and that naturally elevated plasma CORT is the primary mediator of this change. We tested this hypothesis using male house sparrows [*Passer domesticus* (Linnaeus 1758)], a species whose stress response and immune systems are well characterized (Rich and Romero, 2001; Martin et al., 2005, 2006; Kuhlman and Martin, 2010). We assessed the innate immune system by measuring the activity of natural antibodies and complement proteins, which regulate the ability to, respectively, recognize and agglutinate foreign antigens and kill foreign cells (Matson et al., 2005; French and Neuman-Lee, 2012). We measured stress-induced effects on innate immune activity at 10 min and at 120 min after the initiation of the stress response for two reasons. First, we aimed to discern whether these effects were controlled by genomic and/or non-genomic mechanisms based on the assumption that any change in immune activity within 10 min of the stress response initiation resulted from non-genomic mechanisms (Haller et al., 2008). However, it was difficult to predict whether immune activity would be enhanced or suppressed as rapidly as 10 min after the activation of the stress response. Second, we expected that a prolonged stress response would suppress immune activity so as to limit the damage of the innate immune system on the body's tissues. To determine whether naturally elevated plasma CORT was primarily responsible for acute stress-induced changes in immune activity, we measured this activity in pharmacologically adrenalectomized birds (Breuner et al., 2000). If elevated plasma CORT played an essential role in mediating stress-induced effects on immunity, we predicted that pharmacologically adrenalectomized birds would exhibit no change in immune activity during stress.

MATERIALS AND METHODS

We captured 20 adult male house sparrows with mist-nets and baited ground traps in late February 2015 in Phoenix, AZ (33.4°N, 111.6°W; 331 m a.s.l.). All birds had black beaks and, thus, were in reproductive condition (Barfuss and Ellis, 1971). Birds were transported to Arizona State University's Animal Care Facilities, where they were randomly divided into two groups of 10 birds each and housed in identical rooms (*N*=10 per room) under a long-day (13 h:11 h light:dark) photostimulatory photoperiod (Barfuss and Ellis, 1971). Birds were housed individually and were visually isolated from each other, and received *ad libitum* Mazuri Pellet Diet (PMI Nutrition International, Richmond, IN, USA) and water. All procedures were approved by the Arizona State University's Institutional Animal Care and Use Committee and were conducted under Arizona Game and Fish Department scientific collecting permit SP719136.

Stress trials

We investigated (1) the stress-induced effects on innate immune activity at two times during the stress response and (2) whether pharmacological inhibition of CORT production influences these effects.

The experiment consisted in two identical stress trials such that, by the end of the experiment, each bird had experienced experimental stress twice, had received one mitotane and one control (vehicle) injection, and had yielded four blood samples (Fig. 1). For both trials, birds in one group (*N*=10; 10-min group) were exposed to experimental stress for 10 min and birds in another group (*N*=10; 120-min group) were exposed to experimental stress for 120 min. During the first trial, five birds in the 10-min group and five birds in the 120-min group (*N*=5) received mitotane treatment, and the other birds (five in each group) received the control (vehicle) treatment. During the second trial, which began 10 days after completion of the first stress trial, mitotane and control treatments were reversed. One bird died from each group over the course of the experiment.

All trials began at the same time of day (12:00 h) to account for a potential daily rhythm of baseline (BL) plasma CORT (Rich and Romero, 2001). Each bird received one injection (mitotane solution or vehicle) 2 days prior to the beginning of the stress trial because mitotane administration to house sparrows decreases plasma CORT within 36 h of an injection (Breuner et al., 2000). On the day of the stress trial, we removed birds from their home cage, collected a BL blood sample (220 µl) and induced a stress response by placing the bird in a breathable cloth bag and then placing the bag into the bird's cage. At the end of the restraint period, a second (stress-induced) blood sample was collected (220 µl). The volume of blood samples was determined by estimating the minimal volume that was necessary to measure plasma CORT and immune parameters (see below). All blood samples were obtained from the jugular vein using a heparinized microsyringe, and collected within 3 min of

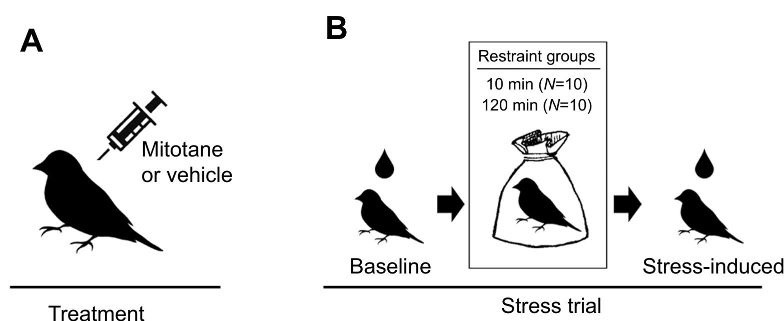

Fig. 1. Experimental design. Adult male house sparrows, *Passer domesticus*, were divided into two groups (*N*=10 each): 10 min and 120 min. Each bird was first treated with either mitotane or vehicle solution (A). Two days later, each bird was exposed to a stress trial (B). We collected a baseline blood sample, restrained the bird for the duration of the bird's group, and collected a stress-induced blood sample. Each bird then rested for 10 days, received the opposite treatment (A) and was exposed to a second stress trial (B).

removing a bird from its cage and within 5 min of entering the room. Samples were immediately placed on ice and were centrifuged within hours of collection. Plasma was then separated and stored at −80°C until assayed.

Mitotane preparation and treatment

Mitotane (#25925, Sigma Aldrich, St Louis, MO, USA) was dissolved in peanut oil (90 mg ml^{-1}). This solution was stored at 4°C for up to 5 days before use and administered into the left pectoral muscle (100 µl, equal to 9 mg per bird per injection). Control injections consisted of 100 µl of peanut oil.

A mitotane injection at the same dose as used here was previously shown to reduce plasma CORT for up to 10 days in adult male house sparrows (Breuner et al., 2000) and mitotane treatment inhibits CORT production in mammals (Chortis et al., 2012) and birds (Jonsson et al., 1994; DuRant et al., 2016). To counter potential problems of glucose mobilization resulting from low plasma CORT and to standardize the feeding regimen, we supplemented all birds, regardless of treatment, with fresh Nektar solution (13 g 100 ml^{-1} 0.9% NaCl in water; Nekton, Germany) daily during the 10 days following injections of mitotane or vehicle.

Plasma corticosterone assay

We measured total plasma CORT with a validated commercial competitive enzyme-linked immunoassay according to the manufacturer's instructions (Enzo Life Sciences, Farmingdale, NY) (Fokidis et al., 2009). House sparrow plasma was diluted 15× in assay buffer containing steroid displacement reagent to dissociate the hormone from plasma binding proteins. Each assay plate included a standard curve and samples were assayed in duplicate. Samples were randomly assigned to assay plates but all four samples from the same individual were assayed on the same plate. The assay sensitivity was 4.90 pg ml^{-1}. The average intra- and interassay coefficients of variation were 4.9% and 13.2%, respectively (N=2 plates).

Hemolysis–hemagglutination assay

We measured the activity of natural antibodies and complement following Matson et al.'s (2005) method. Samples were randomly assigned to 96-well plates, but all four samples from the same individual were assayed on the same plate. Plasma (40 µl) was added to the first column of each plate and then serially diluted in 0.9% phosphate buffered solution (PBS) until the 11th column. The 12th column contained only PBS and served as a negative control. We then added 20 µl of 0.5% whole sheep blood (#SB050, Hemostat Laboratories, Dixon, CA, USA) to each well, covered the plates, and sealed them with Parafilm.

All plates were incubated at 37°C, the incubation temperature that is effective for hemolysis–hemagglutination assay using house sparrow plasma (Martin et al., 2006), for 90 min. Plates were then moved to room temperature and tilted at a 45 deg angle for 20 min. Plates were then scanned for agglutination at 600 dots per inch with a flat-bed scanner (ScanJet 3670, Hewlett-Packard, Palo Alto, CA, USA). After scanning, plates were placed flat at room temperature for 70 min and then scanned again for lysis. Each row of wells was scored for agglutination and lysis by an individual without knowledge of the experimental treatment. High scores reflected high agglutination and lytic activity. All wells were scored in one session to maximize consistency.

Bacterial killing assays

To determine the microbicidal activity of plasma, we used French and Neuman-Lee's (2012) *ex vivo* bacterial killing assay (BKA) method with modifications. *Escherichia coli* (ATCC NO. 8739) was reconstituted from a lyophilized pellet (Epower Assayed Microorganism Preparation; Microbiologics Inc., Saint Cloud, MN, USA) in pre-warmed PBS to make a stock solution of 10^7 colony-forming units (CFU). For each BKA, we prepared a working solution of 10^5 CFU from the stock solution.

We conducted BKAs using 96-well plates and plasma that had not been previously thawed. Each plate included negative (24 µl PBS) and positive controls (18 µl PBS and 6 µl 10^5 *E. coli* CFU). For each sample, we added 7 µl of plasma to 11 µl of PBS and 6 µl of bacteria working solution and assayed each sample in duplicate. Samples were randomly assigned to plates, but all four samples from the same individual were assayed on the same plate. We added 125 µl of Tryptic Soy Broth (15 g broth/500 ml nanopure water; #T8907 Sigma-Aldrich) to all wells and obtained a background reading (300 nm) with a microplate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA). All plates were then incubated at 37°C for 12 h and then read again.

To evaluate the capacity of the plasma to kill *E. coli*, we first subtracted the background reading from the 12 h reading. We then averaged the positive controls and the duplicates for each sample and calculated the percentage of bacteria killed in each well as described by French and Neuman-Lee (2012).

Statistics

We analyzed all data sets using a three-way mixed design ANOVA to examine the effects of the duration of experimental stress (10 min or 120 min), treatment (mitotane or control) and stress (BL or stress-induced) on plasma CORT, agglutination scores, lysis scores and bacterial killing capacity. All data were first tested for normality with the Shapiro–Wilk test. Data sets that could not be normalized by log transformation were ranked before ANOVA (Conover and Iman, 1981). When significant main effects and/or interactions were detected, we used simple main effects to compare specific groups. We compared BL plasma CORT during the first and second trials using a paired Student's t-test to test the potential effect of captivity-associated stress. All statistical analyses were performed with SPSS Statistics 21 (IBM Corporation, New York, NY, USA) and GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). The statistical significance level of all tests was set to P=0.05.

RESULTS

Effects of mitotane treatment, restraint and captivity on plasma CORT

Baseline and stress-induced plasma CORT were lower in mitotane-treated than in control birds ($F_{1,16}$=44.524, P<0.001 and $F_{1,16}$=129.319, P<0.001, respectively; Fig. 2). The duration of restraint did not affect the strength of the stress response ($F_{1,16}$=1.881, P=0.189; Fig. 2). However, mitotane treatment suppressed the stress response: stress-induced plasma CORT was higher than BL plasma CORT in vehicle-treated ($F_{1,16}$=77.314, P<0.001) but not in mitotane-treated birds ($F_{1,16}$=2.248, P=0.153; Fig. 2). Baseline CORT did not differ during the first and second stress trials (t=–0.029, d.f.=17, P=0.977).

Effects of mitotane treatment and restraint on innate immune measures

Bacterial killing capacity changed during the stress response in vehicle-treated birds ($F_{1,16}$=11.959, P=0.003) but not in those receiving mitotane ($F_{1,16}$=4.267, P=0.055; Fig. 2). The duration of experimental stress did not influence how bacterial killing capacity changed during the stress response ($F_{1,16}$=0.023, P=0.882). We also

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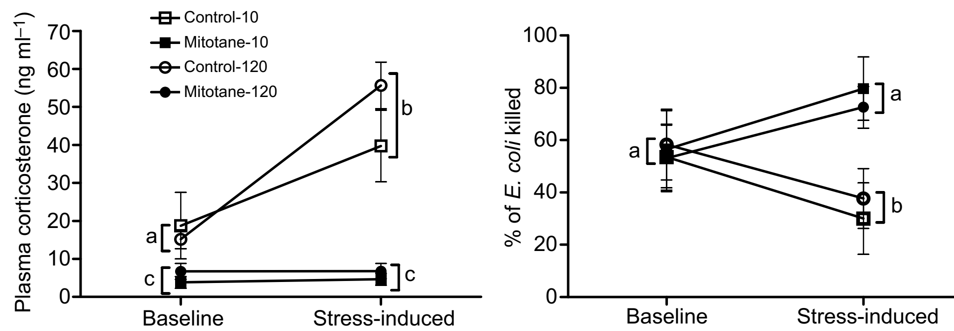

Fig. 2. Baseline and stress-induced levels of plasma corticosterone (left) and bacterial killing capacity (right) of mitotane-treated and vehicle-treated house sparrows. Birds (N=9 per group) treated with mitotane or vehicle were restrained for either 120 min (Mitotane-120 and Control-120, respectively) or 10 min (Mitotane-10 and Control-10, respectively). Letters indicate significant differences (P<0.05) between groups and data are shown as means±s.e.m.

found no evidence for an effect of mitotane treatment on BL bacterial killing capacity ($F_{1,16}$<0.001, P=0.986).

Agglutination scores decreased during the stress response in birds that experienced experimental stress for 120 min ($F_{1,16}$=14.754, P=0.001) but not 10 min ($F_{1,16}$=0.192, P=0.667; Fig. 3). However, this decrease took place only in birds receiving the vehicle injection (vehicle: $F_{1,16}$=40.210, P< 0.001; mitotane: $F_{1,16}$=0.031, P=0.864). There was no effect of treatment on BL agglutination scores ($F_{1,16}$=1.584, P=0.226).

Lysis scores decreased during the stress response in birds receiving the control ($F_{1,16}$=5.508, P=0.032) but not the mitotane treatment ($F_{1,16}$=0.650, P=0.432; Fig. 3). The duration of experimental stress did not influence the change of lysis scores during the stress response ($F_{1,16}$=1.122, P=0.066). Furthermore, mitotane- and vehicle-treated birds had similar BL lysis scores ($F_{1,16}$=1.567, P=0.229).

DISCUSSION

We found in male house sparrows that acute stress is associated with suppression of the innate immune system activity. The onset of this suppression was rapid and the effects were persistent, as shown by the fact that complement-mediated lysis and bacteria killing ability decreased within 10 min of stress exposure and remained low for the next 120 min of this exposure. However, the onset of agglutination, which is mediated by natural antibodies, began between 10 and 120 min of restraint. These results are consistent with the hypothesis that prolonged activation of the stress response inhibits innate immune activity, but do not support the hypothesis that the effects of acute stress on innate immunity change over the duration of the stress response. Furthermore, the results suggest the involvement of both non-genomic and genomic mechanisms. We also found that pharmacological adrenalectomy induced by mitotane administration eliminated the immunosuppressive effects of stress, suggesting that elevated plasma CORT during stress plays an essential role in mediating these effects. To our knowledge, this is the first study demonstrating the necessity of elevating plasma CORT to induce immunosuppression in free-living birds. The findings also indicate that mitotane is an effective agent to investigate relationships between plasma CORT and immune activity.

Stress-induced suppression of innate immune constituents

The effects of acute stress on the innate immune system can be difficult to predict, and investigations of stress-induced effects on innate immune activity in free-living birds have yielded conflicting results. Overall, our results show stress-induced inhibition of innate immune activity and are consistent with previous findings in other free-living birds (Martin et al., 2005; Matson et al., 2006; Cyr et al., 2007; Merrill et al., 2012; Zylberberg, 2015; Davies et al., 2016). They also indicate that the inhibition of complement-mediated activity occurs with a shorter latency than the inhibition of natural antibody-mediated activity. Two other studies have observed differing latencies when investigating the effects of stress on immune activity, but contrary to our findings, these studies showed immunoenhancing effects of stress. In the small ground finch, stress elevated natural antibody-mediated activity at a faster rate than complement-mediated activity (Zylberberg, 2015). In red knots, stress increased phagocytic activity against *Staphylococcus aureus* faster than against *Candida albicans*, and furthermore, did not alter microbicidal activity against *E. coli* (Buehler et al., 2008). Together, these results highlight the importance of measuring immune parameters at various times during the stress response and contribute to accounting for disparities between results from avian studies examining the relationship between stress and the immune system. We propose two explanations for our finding that stress did not result in immunoenhancement. First, this observation may indicate a fixed allocation of resources between reproduction, stress and immunity (Moore and Hopkins, 2009). Supporting this hypothesis, male house sparrows in reproductive condition, such as those in the present study, mount weaker cutaneous immune

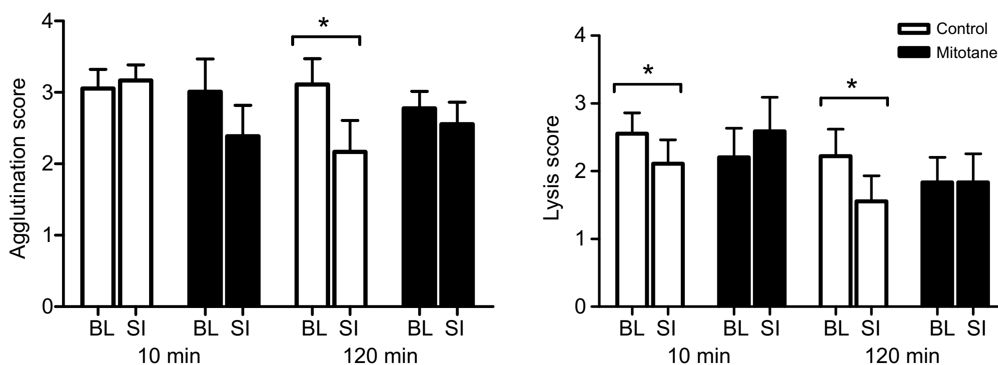

Fig. 3. Baseline and stress-induced levels of agglutination (left) and lysis scores (right) of mitotane-treated and vehicle-treated house sparrows. Birds (N=9 per group) treated with mitotane or vehicle were restrained for either 120 or 10 min. Asterisks indicate significant differences (P<0.05) between groups and data are shown as means±s.e.m. BL, baseline; SI, stress-induced.

responses than molting, non-breeding males even when receiving food *ad libitum* (Greenman et al., 2005; Lee et al., 2006). Second, stress-induced immunosuppression may reflect a transient re-direction of limited resources in preparation for energetically expensive behavior such as flight. For example, innate immune activity is reduced in European starlings, *Sturnus vulgaris*, immediately after prolonged flight but partially recovers within 48 h (Nebel et al., 2012). One such limited resource may be protein availability, as indicated by the fact that low protein stores are associated with reduced activity of constitutive immunity in fasted mallards, *Anas plathyrhynchos* (Bourgeon et al., 2010), and in red knots during migration (Buehler et al., 2010).

The involvement of non-genomic mechanisms in the observed effects of stress on immunity is suggested by the finding that complement-mediated lytic and microbicidal activity decreased within 10 min. To our knowledge, only one other study has investigated rapid stress-induced effects on immune activity in free-living birds. In this study and contrary to the present work, stress for 15 min enhanced agglutination activity (Zylberberg, 2015). These conflicting results may arise from the differing pathogen environment of temperate and tropical birds (Buehler et al., 2008), although stress-induced effects on immune activity may vary even within tropical avian species (Zylberberg, 2015). Despite the disparity, these findings are consistent with the hypothesis that that stress alters immune activity through non-genomic mechanisms. One such mechanism may consist of a decrease in constitutive immunity following loss of blood during collection of the BL sample. We consider this possibility to be unlikely because we did not observe a rapid decrease in agglutination activity. Furthermore, Buehler et al. (2008) found no effect of the number of blood sampling within a short time frame on constitutive immunity. Alternatively, acute stress may stimulate the movement of complement proteins from the blood to other tissues. Mammalian studies have demonstrated stress-induced immunoredistribution of leukocytes (reviewed in Dhabhar, 2009). Leukocytes are also redistributed from the blood into other tissues during long-term captivity in the house sparrow (Kuhlman and Martin, 2010). We are not aware of studies demonstrating this phenomenon in response to restraint, but our results do not exclude this possibility.

In laboratory rodents, an increase in plasma glucocorticoid during stress is a primary inhibitor of immune activity (Sapolsky et al., 2000). Most studies on this subject in free-living species have been correlational and so there is limited support for a causal relationship between elevated glucocorticoids during stress and immunosuppression (Berger et al., 2005; Lindström et al., 2005; Matson et al., 2006; French et al., 2010; Kuhlman and Martin, 2010; Hopkins and Durant, 2011). By manipulating endogenous plasma CORT while controlling for the presence of the stressful stimulus, our results suggest a causal relationship between elevated plasma CORT during stress and the suppression of innate immunity. A negative, causal relationship between these two factors has been also observed in male brown-headed cowbirds (*Molothrus ater*) that were treated with CORT and then restrained for 90 min (Merrill et al., 2012). Our results are also consistent with studies simulating stress by experimentally elevating plasma CORT but not involving exposure to a stressor such as restraint (Martin et al., 2005; Loiseau et al., 2008; Shini et al., 2008). Our findings do not, however, reveal whether elevated plasma CORT directly reduces innate immunity or exerts this effect through other mediators. In Abert's towhees (*Melozone aberti*), the stress-induced suppression of agglutination and lysis scores were not correlated with stress-induced plasma CORT levels (Davies et al., 2016), suggesting that plasma CORT acts indirectly on the innate immune system. With the present data, these observations suggest that stress-induced suppression of innate immune activity is at least indirectly caused by elevated plasma glucocorticoids rather than resulting from exposure to a stressor per se.

We found that the decrease of BL plasma CORT resulting from mitotane administration was not associated with suppression of innate immune activity prior to restraint. BL plasma CORT is thought to facilitate the energetic demands to fuel innate immune activity, although both positive (Merrill et al., 2014) and negative (Zylberberg, 2015) correlations have been found in free-living birds between BL CORT levels and innate immune activity. Our results, while not supporting this hypothesis, suggest mechanisms that may be involved in the observed effects. In house sparrows, low BL plasma CORT acts on cells by binding to high affinity mineralocorticoid receptors (MR) whereas high plasma CORT, such as during stress, acts by binding to high affinity MR as well as low affinity glucocorticoid receptors (GR) (Lattin et al., 2011). Accordingly, stress-induced immunosuppression may be mediated primarily through GR. This hypothesis would explain the lack of relationship between BL plasma CORT and innate immune activity, but warrants further research. Indeed, we are not aware of studies investigating the role of GRs during stress-induced changes in immune activity in adult free-ranging birds. Research on this subject and using specific GR antagonists may help clarify this relationship.

Conclusions

Our results suggest that elevated plasma CORT is a primary component of the stress response that inhibits natural antibody and complement-mediated immune activity. The rapid stress-induced suppression of complement-mediated activity is especially intriguing given the sparse data on stress-induced immune effects within a non-genomic time frame in free-living birds. Our results also highlight the necessity to measure immunological parameters at various times during the stress response, as it appears that the activation of the stress response does not act on all constituents of the innate immune system with the same latency. Our findings do not reveal the involvement of any specific CORT receptor type or whether plasma CORT acts directly or indirectly to suppress the activity and/or concentration of complements and natural antibodies in the plasma. However, given that the reduction of BL plasma CORT resulting from mitotane treatment was not associated with suppression of innate immune activity prior to restraint, our results suggest that CORT acts through GRs rather than MRs to reduce innate immune activity. Furthermore, the rapid onset and persistence of stress-induced immunosuppression implies a role for both non-genomic and genomic pathways in causing the observed immune effects of stress.

Acknowledgements

We thank C. Das and R. Johnson for their assistance with the study, S. French (Utah State University) for assistance with the bacterial killing assay, and M. Angilletta, K. McGraw and K. Sweazea (Arizona State University) for access to laboratory facilities and equipment. We thank the Badman family for allowing us to capture birds on their property.

Competing interests

The authors declare no competing or financial interests.

Author Contributions

S.G. and P.D. designed the experiments and drafted the manuscript. S.G. administered all of the treatments and collected all blood samples. S.G. and C.S. performed the corticosterone and immune assays. S.G. performed all the statistical analyses.

Funding
This project was funded by grants awarded by the School of Life Sciences at Arizona State University to S.G.

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