General and Comparative Endocrinology 193 (2013) 185–192

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Acute stress during ontogeny suppresses innate, but not acquired immunity in a semi-precocial bird (*Larus delawarensis*)

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ARTICLE INFO

Article history:
Received 1 February 2013
Revised 11 July 2013
Accepted 14 August 2013
Available online 27 August 2013

Keywords:
Stress response
Natural antibodies
Complement
Immunoglobulin-Y
Development
Corticosterone

ABSTRACT

Wild animals often encounter adverse conditions, and in response, activate their hypothalamic–pituitary–adrenal (HPA) axis. To date, work examining the development of the stress response has focused on altricial species, with little work focusing on species with other developmental patterns. Additionally, the effects of acute stress on indices of innate and adaptive immunity have been little studied in birds, particularly during development. We examined the ontogeny of the stress response in the semi-precocial ring-billed gull (*Larus delawarensis*). At hatch, 10, and 20 days post-hatching, chicks underwent a standardized handling stress protocol, with blood samples taken within 3 min of, and 30 min after, initial disturbance. Levels of corticosterone (CORT), natural antibodies (NAb), complement activity, and immunoglobulins (IgY) were assessed in plasma samples. In contrast with altricial species, ring-billed gull chicks had detectible CORT levels at hatch, and were able to mount a stress response. At all ages, acute handling stress depressed NAb levels and complement-mediated lysis, but not IgY levels. IgY levels were higher in two chick broods than three chick broods, suggesting levels are determined in part by resource dependence. Our data provide insight into the development of the stress response and immune function in a colonial waterbird species, in which chicks are mobile shortly post hatch, and subject to aggression and possible injury from nearby adults.

1. Introduction

In the wild, animals are exposed to various stressors, including unpredictable environmental conditions, such as severe weather (Romero et al., 2000; Talloen et al., 2010; Wingfield et al., 1983) and reduced resource availability (Honarmand et al., 2010; Kitaysky et al., 2001, 1999a, 1999b). In response to such conditions, vertebrates activate the hypothalamic–pituitary–adrenal (HPA) axis and glucocorticoid (GC) secretion increases. Although the stressor-induced GC levels can be an indicator of fitness and survival (reviewed in Blas et al. (2007), Breuner et al. (2008)), long-term exposure to elevated GCs can have persistent negative effects, including suppressed immunity and decreased body condition (Heath and Dufty, 1998).

While the majority of work on stress in birds has focused on adult responses to stressful environments (e.g. Hau et al., 2010; Holberton, 1999), there is increasing interest in the adrenocortical response during ontogeny (Love et al., 2003, 2009). Most studies have focused on altricial species whose chicks receive extensive parental care and are confined to the nest (e.g., Rivers et al., 2012; Wada et al., 2007; Wada et al., 2009). During early post-hatching development in altricial species, circulating GCs are low and the HPA axis is hyporesponsive to stressors (Wada et al., 2009). Because GCs promote protein catabolism, the hyporesponsive period in altricial birds likely promotes protein anabolism and allows the chick to continue development (Wada et al., 2009), as occurs in mammals (Sapolsky and Meaney, 1986). The reactivity of the HPA axis in altricial birds increases throughout development, and individuals display an adult-like stress response when near independence (Wada et al., 2009).

In contrast to altricial species, little is known about the ontogeny of the HPA axis in non-domesticated birds with semi-precocial or precocial developmental patterns. Semi-precocial chicks hatch covered in down and can move from the nest within a few days of hatching. Semi-precocial chicks have less developed locomotor activity than precocial chicks, and require strong nest attendance by parents and completely depend on parents for food (Starck and Ricklefs, 1998). Because semi-precocial chicks are more active and mobile than nest-bound altricial chicks, they may encounter a greater range of stressors (e.g., in gulls, chicks can face predators and/or aggression from neighbors in colonial species (Ryder, 1983). As such, semi-precocial chicks should have a robust stress response from an early age. Nonetheless, irrespective of the mode

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http://dx.doi.org/10.1016/j.ygcen.2013.08.007

of development, growth and tissue maturation can be inhibited by GC secretion (Schmidt et al., 2012; Spencer et al., 2003; Spencer and Verhulst, 2007), thus resulting in the existence of potential trade-offs between the need for a robust stress response and chick growth.

Chronic elevation of GCs via activation of the HPA axis can have negative effects beyond growth suppression (Sapolsky et al., 2000). For example, chronic elevation of corticosterone (CORT, the dominant GC in birds) has been linked to a decreased T-cell mediated immune response in chicks (Saino et al., 2003) and adults (Martin et al., 2005), as well as decreased antibody production (Stier et al., 2009). In contrast, transient, brief periods of stress may be immunoenhancing, promoting the first line of immune defence, such as the inflammatory response (reviewed in Martin (2009)). However, relatively little is known about how acute stress affects other aspects of the innate or adaptive immune system in chicks (but see Buehler et al., 2008; Matson et al., 2006; Merrill et al., 2012).

In this study, we characterized the relationship between baseline and stressor-induced CORT levels on immune function during ontogeny in the semi-precocial ring-billed gull (*Larus delawarensis*). The ontogeny of the stress response is less well understood in semi-precocial species, than in altricial species (see Love et al., 2003; Wada et al., 2009) or domesticated precocial species such as chickens (Freeman and Flack, 1980). Moreover, studies examining the effects of environmental stressors on the immune system during ontogeny have focused primarily on chronic (Stier et al., 2009) (De Coster et al., 2010) rather than acute stressors.

Ring-billed gulls are a colonial waterbird, where chicks are semi-precocial; they hatch covered in down, but must be fed by parents. Females typically lay 3 eggs, and hatching is often asynchronous. Asynchronous hatching can create a size hierarchy, which may allow parents to preferentially feed older chicks at the expense of later hatched siblings (Hébert and McNeil, 1999), which often suffer increased mortality rates (Woulfe, 1989). Within 5 days post-hatch, chicks can wander from the nest site, but may be subject to aggression from neighboring adults (Brown, 1998). As a result of the behavior of ring-billed gull chicks, we made the following predictions: (1) chicks will hatch with detectible levels of CORT, and will be capable of elevating CORT above baseline in response to an acute stressor because chicks are semi-precocial and mobile shortly after hatch; (2) Because of their mobility, chicks may encounter aggression from neighboring adults, and as a result will hatch with innate and adaptive immune function, as indicated by detectible levels of natural antibodies (NAb) and complement-mediated lysis (complement) (indices of innate immunity), and immunoglobulin-Y (IgY, an index of adaptive/acquired immune function); (3) An acute stressor will increase levels of NAb and complement activity, but suppress levels of IgY. This is expected because short-term stress may enhance innate immune function, but suppress maturation of immune cells (reviewed in Martin (2009)); finally, (4) CORT levels and indices of immune function will vary with brood size because these metrics may be influenced by food availability during ontogeny.

2. Methods

2.1. Field site and subjects

Field work was conducted between April and June 2007 on Pier 27 in Hamilton Harbour in Burlington, ON, Canada (43° 15′ 19″ N, 79° 52′ 23″ W) under a Canadian Wildlife Services Permit (CA0219) and Trent University Animal Care Permit (08013). Ring-billed gulls typically lay 3 egg clutches, over a 3–5 day period. On average, eggs are incubated over a 25-day period, and then the chicks hatch asynchronously over a 2 day period. Chicks have finished the sigmoidal period of growth by 22 days, reaching 70% of adult size, and typically fledge at ~35 days (Pollet et al., 2012).

Clutch initiation was monitored and recorded daily in the mornings. Upon discovery of a new egg in a nest, fresh egg mass, length and width were measured, and each new egg was marked with a non-toxic pen to track laying sequence. Nests were fenced-in upon clutch completion. Fencing was ca. 75 cm in diameter; wide enough to allow chicks to wander a short distance, but not wander so far that we could not collect a blood sample within 3 min of disturbance. Upon hatching, chicks were marked with a non-toxic dye (Nyanzol) on either the head or rump, or left unmarked for identification until 5 days of age when each chick received a web tag on the left foot. Chicks were later banded with an aluminum leg band at 10 days of age.

2.2. Growth

Beginning at hatch, chicks were removed from the nest every 5 days for morphological measurements. Because hatching in the gull colony is not strongly synchronous, it was not possible to measure all chicks on the same days. Accordingly, for each age class we measured morphology and/or blood sampled birds within a 3-day window (hatch: 0–3 days, 5 days old: 5–7 days, 10 days old: 9–11 days, 15 days old: 15–17 days, and 20 days old: 19–21 days). Measurements of mass, exposed culmen, back of cranium to tip of upper bill, depth at gonys, metatarsus and wing chord were taken and chicks were returned to the nest.

2.3. Stress response

At post-hatch days 0–3 (considered hatch), 9–11 (considered 10 days old), and 19–21 (considered 20 days old), birds underwent a standard handling stress protocol (Wingfield et al., 1995). Briefly, between the hours of 1600 and 2000, chicks were bled within 3 min of initial disturbance of the nest. After blood sampling, morphological measurements were taken, and birds were placed in a breathable cloth bag until 30 min of initial disturbance. At 30 min after initial disturbance, another blood sample was taken, to measure stressor-induced corticosterone levels (stressor-induced CORT levels are reached by 30 min in *Charadiiformes* (Reneerkens et al., 2002). Chicks were then returned to the nest. Samples were placed in a cooler and brought back to McMaster University for processing within 4 h of collection. Blood samples were spun at 15700 g for 3 min. Plasma was separated from red blood cells and both were stored at −20 °C during the field season, and then transferred to −80 °C until further analysis.

2.4. Molecular sex determination

Sex of ring-billed gull chicks was determined via amplification of the CHD genes using polymerase chain reaction (PCR) using the P2/P8 primer set (Griffiths et al., 1996). Chick sex was determined from frozen red blood cells. Briefly, DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit following manufacturer's protocols. PCR amplification on extracted DNA was performed using the P2 (5′-TCTGCATCGCTAAATCCTTT) and P8 (5′-CTCCCAAGGATGAGRAAYTG) primer set. PCR products were separated on 3% agarose gels with ethidium bromide and visualized under UV light. Positive controls of adults of known sex ($n = 2$ per sex) were used. Chicks were identified as male (CHD-Z gene amplification only) or female (CHD-Z gene and CHD-W gene amplification) (Chin et al., 2005).