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Prolactin is related to individual differences in parental behavior and reproductive success in a biparental passerine, the zebra finch (*Taeniopygia guttata*)

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ABSTRACT

Variation in parental care can lead to important fitness consequences. The endocrine system is known to regulate physiological and behavioral reproductive traits that are important contributors to lifetime reproductive success. However, the hormonal basis of variation in avian parental care is still not well understood. Plasma prolactin (PRL) concentrations are generally high during post-hatch parental care in birds, and may be a candidate mechanism that regulates variation in parental care and other reproductive success outcomes. Here we analyze the relationship between PRL, parental behavior (chick brooding and feeding) and reproductive success outcomes (clutch size, number of chicks hatched, and chick survival) for the first time in the zebra finch (*Taeniopygia guttata*). Birds were given cabergoline, a dopamine agonist traditionally used to lower prolactin in mammals, or vehicle in their food. Cabergoline had no effect on prolactin concentrations, but across both groups we found that PRL is positively correlated with parental behavior, number of chicks hatched, and chick survival, but not clutch size. Results from this study will inform hypotheses and predictions for future manipulation studies which test for a causal role for PRL in parental traits.

1. Introduction

Variation in parental care can play a critical role in determining an offspring's phenotype and survival (Royle et al., 2012), and so has important fitness consequences. Therefore, it is important to understand the underlying sources and mechanisms behind this variation in order to gain a fuller understanding of the evolution of this reproductive behavior. The neuroendocrine systems are known to regulate various reproductive traits that make up aspects of an individual's reproductive phenotype, as well as to coordinate physiological and behavioral processes in response to internal and external cues to maximize fitness. As such, hormonal measurements have become an increasingly popular tool to predict reproductive effort and success in free-living birds. However, our understanding of the physiological and hormonal basis of phenotypic variation in reproductive traits and behaviors that contribute to lifetime fitness is still rudimentary (Williams, 2012). A more in-depth understanding of the dynamics of hormone-behavior relationships will inform hypotheses and predictions for future experimental manipulations which test for a causal role of hormonal contributions to individual variation in parental care.

One potential source of individual variation in parental care and other reproductive traits may come from prolactin (PRL) during breeding. Plasma PRL is significantly elevated above the low, non-breeding baseline levels during late incubation and post-hatch care in many birds that hatch altricial young (Angelier et al., 2016; Buntin, 1996; Smiley and Adkins-Regan, 2016; Sockman et al., 2006) and is thought to play a significant role in promoting the onset of parental behavior (Angelier et al., 2016). Because of this pattern, researchers have become increasingly interested in using PRL as hormonal predictor of individual variation in reproductive success and parental investment in free-living birds, particularly in passerines. For example, higher pre-breeding baseline PRL concentrations correlate positively with earlier laying dates in free-living great tits (*Parus major*; Ouyang et al., 2013) and earlier egg laying dates and total numbers of fledglings for the breeding season in free-living house sparrows (*Passer domesticus*; Ouyang et al., 2011). PRL has also been found to be positively correlated with hatching success in wild common terns (*Sterna hirundo*; Riechert et al., 2014), nestling feeding and provi-

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sioning rates in house finches (*Carpodacus mexicanus*; Badyaev and Duckworth, 2005; Duckworth et al., 2003), black-legged kittiwakes (*Rissa tridactyla*; Chastel et al., 2005), red-cockaded woodpeckers (*Picoides borealis*; Khan et al., 2001), Florida scrub-jays (*Aphelocoma coerulescens*; Schoech et al., 1996) and Harris' hawks (*Parabuteo unicinctus*; Vleck et al., 1991) and nestling weight in mourning doves (*Zenaida macroura*; Miller et al., 2009). Conversely, low PRL is associated with poor environmental conditions, poor body condition, breeding failure, and nest abandonment (reviewed in Angelier and Chastel, 2009; Angelier et al., 2016). However, it is still unknown whether variation in reproductive success is a result of different PRL concentrations altering parental care behavior, or whether the variation in PRL concentrations observed is a result from cues from external breeding stimuli.

There is evidence for a bidirectional relationship between elevated PRL and parental behavior, and they likely feedback onto one another reciprocally. For instance, maintenance of elevated PRL during incubation depends on physical contact with the nest and eggs in avian species that hatch precocial young, such as galliformes (poultry) and anseriformes (ducks), (reviewed in Angelier et al., 2016; Buntin, 1996; Sockman et al., 2006). Removal of the nest or eggs during incubation results in a decline in PRL, while replacing the nest or eggs after removal reinstates elevated levels of PRL (reviewed in Buntin, 1996). Likewise, in avian species that hatch altricial young, PRL is highest immediately post-hatch, when the most intensive parental care occurs. Experimentally replacing chicks with younger ones can prolong the period of elevated PRL, while replacing chicks with older ones can truncate the period of elevated PRL (reviewed in Buntin, 1996). Taken together, elevated PRL may be necessary to show parental behavior, but this elevation may depend on breeding stimuli, and possibly other external and internal conditions, such as weather and body condition.

In order to begin analyzing the relationship between PRL, parental behavior, and reproductive success further, we measured plasma PRL concentrations on day two post-hatch and related them to variation in parental behavior and other reproductive success measures for the first time in male and female zebra finches (*Taeniopygia guttata*). We, and others, have shown that plasma PRL concentrations are significantly elevated above the non-breeding baseline during late incubation and during early post-hatch care in male and female zebra finches (Christensen and Vleck, 2008; Smiley and Adkins-Regan, 2016). Males and females are socially monogamous and contribute roughly equally to nest building, egg incubation, and post-hatch chick care (Zann, 1996). In addition, parental behavior between breeding partners tends to be well synchronized (Mariette and Griffith, 2012; Van Rooij and Griffith, 2013). Thus, we hypothesized that PRL would be correlated with the amount of parental care behavior displayed immediately post-hatch. In addition, since breeding stimuli, such as eggs and chicks, also appear to influence or maintain elevated PRL concentrations, we hypothesized that PRL would be positively related to other reproductive success measures including clutch size, number of chicks hatched, and chick survival to fledging. Results from this study will inform hypotheses and predictions for future manipulation studies which test for a causal role for PRL in parental traits.

2. Methods

2.1. Subjects

Subjects were 12 zebra finches (6 males and 6 females) that were bred in the lab (Cornell University, Ithaca, NY). All subjects were reproductively mature adults, but age and reproductive history were unknown for most subjects. All birds were kept on a 14:10 light:dark schedule, in a temperature and humidity controlled room. Birds were identified by a unique sequence of colored leg bands and one silver metal leg band engraved with a unique number. Prior to the start of the experiment, subjects were housed in sex-specific aviaries (0.94 m × 0.76 m × 0.94 m) with seed, grit, cuttlebone, and water available *ad libitum*.

2.2. Study design

2.2.1. Breeding pairs

Four of the 12 subjects (i.e., two pairs) were previously established pairs from our lab's breeding colony. The other eight subjects were pairs that formed in social aviaries that housed four males and four unfamiliar females for one week prior to the study. Daily 30-min behavioral observations took place during this week to determine which birds were paired (see Smiley et al., 2012 and Vahaba et al., 2013 for Section 2). Once pairs were determined, each pair (including the two established pairs) were moved to separate testing cages (0.6 m × 0.4 m × 0.35 m), each equipped with a nest box, and nesting material, seed, grit, cuttlebone, and water available *ad libitum*. Testing cages were housed in two different rooms to allow for blood sampling across multiple subjects on the same day if needed (see Section 2.2.4. for details). Daily nest checks were performed to look for eggs and chicks in order to monitor the breeding status for each pair. Incubation typically lasts for 14 days (Zann, 1996). Chicks rely on parental brooding for thermoregulation for at least the first seven days of life and rely on parental feedings for 16–18 days post-hatch, which is around the time that they fledge from the nest (Zann, 1996). Offspring continue to rely on parental feeding for some time after fledging, but are fully transitioned to self-feeding by 30–40 days post-hatch (Zann, 1996).

2.2.2. Cabergoline manipulation

The study was originally intended to be a pilot study to look at the effects of orally administered cabergoline, a potent and orally active dopamine D2 receptor agonist in mammals (Kvernmo et al., 2006), on circulating prolactin levels and parental behavior. Pairs were evenly divided into cabergoline treatment or vehicle groups, but cabergoline treatment did not affect PRL concentrations (see Section 3.1.), so the two groups were combined for behavioral analysis in this study. Briefly, if females laid one egg each day for four consecutive days, pairs were determined to have reached breeding status. Incubation was recorded as beginning on the first day an egg appeared in the nest. Egg viability was checked throughout the incubation cycle by candling eggs with flashlights. All 6 nests had at least one fertile egg in their nest prior to treatment. Beginning on day 12 of incubation, half of the pairs ($n = 6$ birds) received 0.05 ml of cabergoline (Sigma–Aldrich C0246; dose = 0.25 mg/kg body weight based on Brooks et al., 2005) dissolved in fractionated coconut oil on top of 1.3 g of hardboiled egg. The other half of the pairs ($n = 6$ birds) received 0.05 ml of the vehicle alone (control) on top of 1.3 g of hardboiled egg. Each member of the pair received the same treatment. Treatments were randomly assigned to pairs. All subjects received five daily doses of their assigned treatment, approximately 24 h apart. Treatments were administered each morning during the last three days of incubation (incubation days 12, 13, 14) and during the first two days of post-hatch care. Seed dishes were removed at this time to encourage egg eating and returned two hours later. All of the egg was typically consumed during this two-hour period. Parental behavior was recorded on days one and two post-hatch in the mornings after the egg was provided (see Section 2.2.3. for details). On the last day of treatment (day two post-hatch) a blood sample was taken three hours after birds were given the egg mixture (see Section 2.2.4. for blood sampling methods).