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Effects of water restriction on reproductive physiology and affiliative behavior in an opportunistically-breeding and monogamous songbird, the zebra finch

Nora H. Prior [b,*], Sarah A. Heimovics [a], Kiran K. Soma [a,b,c]

[a] *Department of Psychology, University of British Columbia, Vancouver, BC, Canada*
[b] *Department of Zoology, University of British Columbia, Vancouver, BC, Canada*
[c] *Graduate Program in Neuroscience, University of British Columbia, Vancouver, BC, Canada*

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ABSTRACT

Wild zebra finches form long-term monogamous pair-bonds that are actively maintained year-round, even when not in breeding condition. These desert finches are opportunistic breeders, and breeding is highly influenced by unpredictable rainfall. Their high levels of affiliation and complex breeding patterns make zebra finches an excellent model in which to study the endocrine regulation of affiliation. Here, we compared zebra finch pairs that were provided with water ad libitum (control) or water restricted. We examined (1) reproductive physiology, (2) pair-maintenance behaviors in several contexts, and (3) circulating and brain steroid levels. In females, water restriction profoundly reduced largest ovarian follicle size, ovary size, oviduct size, and egg laying. In males, water restriction had no effect on testes size but decreased systemic testosterone levels. However, in the hypothalamus, local testosterone and estradiol levels were unaffected by water restriction in both sexes. Systemic and local levels of the androgen precursor dehydroepiandrosterone (DHEA) were also unaffected by water restriction. Lastly, in three different behavioral paradigms, we examined a variety of pair-maintenance behaviors, and none were reduced by water restriction. Taken together, these correlational data are consistent with the hypothesis that local production of sex steroids in the brain promotes the expression of pair-maintenance behaviors in non-breeding zebra finches.

Introduction

Affiliative, pro-social behaviors are expressed in a wide range of contexts, including courtship, pair-bonding, parental behavior, reconciliation, coordination of group movements, and cooperation (Abartz and Hollander, 2006; Buck, 2002; Carter, 1998; Pellegrini, 2008; Penner et al., 2005). These behaviors include physical contact, allopreening or grooming, and vocal communication (Elie et al., 2010, 2011a; Penner et al., 2005; St-Pierre et al., 2009). The motivation to engage in affiliative behaviors is physiologically regulated (Buck, 2002; Goodson et al., 2005; Lim and Young, 2006; Robinson et al., 2005), and engagement in these behaviors has physiological effects on individuals (Kikusui et al., 2006; Neumann, 2009). While affiliative behaviors are expressed in various contexts, research examining the physiological regulation of these behaviors has been largely restricted to parental behaviors and pair-bond formation in monogamous animals.

Zebra finches are an excellent model in which to study affiliation and its endocrine regulation. They are gregarious and have a large repertoire of affiliative behaviors (Birkhead et al., 1988; Elie et al., 2010; Zann, 1994, 1996). As juveniles, they engage in these behaviors with their cohorts and parents. After sexual maturity, however, they engage in these behaviors almost exclusively with their pair-bonded partner (Birkhead et al., 1988). These long-term pairs are socially and sexually monogamous, and the pair maintains this bond year-round (Birkhead et al., 1988, 1990; Griffith et al., 2010; Zann, 1994).

Zebra finches are opportunistic breeders and integrate many environmental cues to time breeding in arid habitats, including water availability, food availability, presence of green grasses, and photoperiod (Perfito et al., 2007, 2008; Zann, 1994). Water availability is one of the most important cues for wild zebra finches, and water restriction can bring captive male zebra finches out of breeding condition (Morton, 2009; Perfito et al., 2008; Vleck and Priedkalns, 1985). The hormonal profile of non-breeding zebra finches has not been fully characterized, but evidence to date suggests that circulating sex steroids are reduced in non-breeding zebra finches (Perfito et al., 2006, 2007).

While courtship and pair-bond formation in zebra finches have been well studied, the *maintenance* of pair bonds has not. Courtship behaviors such as male song and sexual displays are regulated by sex steroids (Arnold, 1975; Harding and Rowe, 2003; Hill et al., 2005). These behaviors are also important in pair-maintenance. Classically, sex steroids have been thought to be produced in the gonads and then travel to the brain to regulate behavior. However, there is now abundant evidence that sex steroids can also be produced locally in the brain, either *de novo* from cholesterol or from circulating prohormones such as

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[*] Corresponding author at: 2136 West Mall, University of British Columbia, Vancouver, BC, Canada V6T 1Z4.
E-mail address: nhprior@zoology.ubc.ca (N.H. Prior).

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dehydroepiandrosterone (DHEA) (Balthazart and Ball, 2006; Forlano et al., 2006; Schlinger and Remage-Healey, 2012). Behaviors that are regulated by gonadally-produced sex steroids during the breeding season can be regulated by neurally-produced sex steroids during the non-breeding season, when gonadal production of sex steroids is low or absent (Pradhan et al., 2010; Soma et al., 1999, 2000).

Taken together, these studies suggest that pair-maintenance behaviors in zebra finches might be regulated by gonadally-produced sex steroids while pairs are in breeding condition and regulated by neurally-produced sex steroids while pairs are in non-breeding condition. As a first step towards testing this hypothesis, we examined the effects of water restriction on (1) male and female reproductive physiology, (2) pair-maintenance behaviors in a variety of behavioral paradigms, and (3) circulating and brain levels of estradiol, testosterone, and DHEA.

Materials and methods

Subjects

These experiments were carried out under a University of British Columbia Animal Care Committee protocol and followed the guidelines of the Canadian Council on Animal Care. Subjects were adult (>120 d old) captive zebra finches housed in a colony maintained on a 14:10 h light:dark cycle with an average temperature of 22 °C and an average relative humidity of 31%. All zebra finches had ad libitum access to seed (50/50, Panicum millet/white millet, Just For Birds, Langley BC), cuttlefish bone, and grit. Prior to experimental water restriction, all subjects had ad libitum access to water. Male–female dyads were housed together in cages (38 1/2″ × 19 3/4″ × 19″, Corner's Cages) that had a nestbox (5 1/2 ″ × 5 1/2 ″ × 7 1/2 ″) and a center groove into which a divider could be placed. Dyads were housed together for a minimum of 2 months prior to the start of the experimental manipulation. All pairs engaged in affiliative, courtship, and/or nesting behaviors, and were thus considered pair-bonded.

Pairs were then assigned to one of two treatment groups: control (CON, n = 10 pairs) or water restriction (WR, n = 11 pairs). Treatment groups were counterbalanced with respect to the number of eggs laid and chicks hatched per pair during the previous 2 months. Water-restricted subjects were given decreasing amounts of water over the course of 5 weeks, to a minimum of 1 mL per subject per week, which they continued to receive for the duration of the experiment (Table 1). Water-restricted subjects always had access to empty water towers. When water was administered to the water-restricted pairs, a specific amount of water (Table 1) was added to the water tower for a limited period of time (30 to 120 min), and the amount of water consumed was estimated to the nearest 0.25 mL by measuring (and removing) the remaining water with a pipette (Table 1). Control pairs received water ad libitum from their water towers. After the start of the experimental manipulation, all eggs laid were removed from all pairs within 48 h of laying, to prevent parental behavior from being a confound.

General timeline

A timeline for the experiment is shown in Fig. 1. To assess the effects of water restriction on baseline behaviors and circulating steroid levels, Baseline Behavior Sessions were recorded and blood samples were collected before (Pre) and after (Post) water restriction (Fig. 1). Next, we conducted two behavioral tests that elicited pair-maintenance behaviors under different conditions: the ‘Partner Preference Test’ and the ‘Partner Reunion Test’ (see below for details). Immediately following the Partner Reunion Test, blood and brain tissue were collected for quantification of circulating and brain steroid levels (Fig. 1).

Baseline behavior and circulating steroid levels

Baseline behaviors were assessed during two 20-min sessions (40 min total) both before (Pre) and after (Post) water restriction. Pairs were recorded in their home cages in the colony room, between 09:00 and 13:00 h, using a digital camcorder.

To measure the effects of water restriction on circulating steroid levels under normal conditions, blood samples were collected from the brachial vein before (Pre) and after (Post) water restriction (Fig. 1). The male and female of each pair were caught simultaneously from their home cage, ~2 d after the second Baseline Behavior Session. Approximately 150 μL of blood was collected into heparinized capillary tubes within 10 min (6.8 ± 0.2 min) of entering the colony room and stored on wet ice. After centrifugation (10 min at 10,000 g), plasma was collected and stored at −20 °C.

Partner Preference Test and Partner Reunion Test

Both the Partner Preference Test and the Partner Reunion Test took place in a separate testing room (i.e., not the colony room). Between 11:00 and 13:00 h on the day prior to testing, the pairs were moved to a testing room.

For the Partner Preference Test, the home cage was placed in between two smaller stimulus cages. Opaque partitions separated the stimulus cages and the home cage. The male or female in the pair was randomly assigned to be the focal animal, and the other individual was the partner stimulus. The pair was separated immediately prior to lights out (21:00 h), the night before the test. The focal animal remained in the home cage, and the partner stimulus was placed in one of the stimulus cages. At the same time, a novel stimulus individual was placed in the other stimulus cage. Note that the novel stimulus was the same sex and in the same condition (control or water restricted) as the partner stimulus. The sides of the partner and novel stimuli were counterbalanced between the two treatment groups. The following morning, at the start of the test, the opaque partitions were removed, and behavior was recorded for 20 min using a digital camcorder. Note that during the overnight separation, the pair remained in acoustic contact, allowing them to maintain their pair bond (Zann, 1996). Previous studies have used longer separation periods, without disruption of the pair bond (Remage-Healey et al., 2003).

For the Partner Reunion Test, the pairs were again separated immediately prior to lights out (21:00 h), the night before the test. The male and female were placed on opposite sides of the home cage, and they were physically and visually isolated by a wire partition and an opaque partition, which were both inserted into a groove in the center of the cage. The following morning, at the start of the test, the opaque partition only was removed (the wire partition remained in place), so the pair was physically but not visually isolated for 10 min. Next, the wire

Table 1
Water consumption by water-restricted female (n = 11) and male (n = 11) zebra finches.

Week	1	2	3	4	5	6	7	8
Water received	3 × 4 mL	2 × 3 mL	2 × 2 mL	1 × 2 mL	1 × 1 mL	1 × 1 mL	1 × 1 mL	1 × 1 mL
Water consumed, females (mL)	5.5 ± 0.41	2.6 ± 0.22	2.5 ± 0.2	1.2 ± 0.08	0.8 ± 0.02	0.8 ± 0.03	0.7 ± 0.05	0.7 ± 0.06
Water consumed, males (mL)	5.2 ± 0.36	2.9 ± 0.22	2.5 ± 0.14	1.2 ± 0.06	0.8 ± 0.05	0.8 ± 0.01	0.6 ± 0.04	0.7 ± 0.06

Note: Water received is given in number of times per week × volume at each “watering” (e.g., in week 1, individuals received 4 mL of water on 3 separate days).