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Acute and chronic effects of an aromatase inhibitor on pair-maintenance behavior of water-restricted zebra finch pairs

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ABSTRACT

Zebra finches are highly social songbirds that maintain life-long monogamous pair-bonds. They rely heavily upon these pair-bonds to survive their ever-changing and unpredictable habitat in the Australian desert. These pair-bonds are maintained via a large repertoire of affiliative behaviors that for most of an individual's life are predominately associated with pair maintenance. Water restriction reduces circulating testosterone levels in male zebra finches and the size of the ovary and oviduct in female zebra finches, but water restriction has little or no effects on pair-maintenance behaviors and local levels of testosterone and estradiol in behaviorally-relevant brain regions. These data suggest that in water-restricted zebra finches, local synthesis of testosterone and estradiol in the brain may support the expression of pair-maintenance behaviors. Here, we directly test whether pair-maintenance behaviors are regulated by estradiol, acting via non-genomic or genomic mechanisms, in water-restricted (i.e., non-breeding) zebra finches. In two experiments, subjects were treated with an aromatase inhibitor (fadrozole) either acutely or chronically, and a variety of pair-maintenance behaviors were quantified. Additionally, we quantified the effect of acute fadrozole treatment on brain and circulating estradiol and testosterone levels. Acute fadrozole administration rapidly decreased estradiol levels in the circulation and brain of males and also rapidly increased testosterone levels in the circulation and brain of both males and females. However, neither the acute nor chronic fadrozole treatment decreased pair-maintenance behaviors. In one case, acute fadrozole treatment promoted affiliation. These data suggest that pair-maintenance behavior in non-breeding zebra finches is not promoted by estradiol acting via either non-genomic or genomic mechanisms.

1. Introduction

Zebra finches (*Taeniopygia guttata*) are highly social songbirds: they live in large groups and maintain life-long pair-bonds. Pairs are both socially and sexually monogamous and actively maintain their bond regardless of breeding condition (Birkhead et al., 1988; Zann, 1996). Native to the Australian deserts, zebra finches rely heavily upon their pair-bonds to survive the ever-changing and unpredictable habitat. Pairs coordinate almost all of their activities, including foraging, incubation, feeding nestlings, and feeding fledglings (Zann, 1994; Dunn and Zann, 2010; Mariette and Griffith, 2012). More importantly, highly synchronized pairs are more efficient parents and fledge more chicks (Mariette and Griffith, 2012). The pair-bonds are supported by a complex suite of behavioral and physiological mechanisms, which are only beginning to be understood.

The longevity of these pair-bonds is maintained through a large repertoire of affiliative behaviors, including clumping, allopreening, close proximity, coordination and vocalizations (Zann, 1996; Elie et al., 2010; Prior et al., 2013). Juveniles engage in affiliative behaviors with cohorts and parents. When zebra finches reach sexual maturity, these behaviors are used in courtship and pair-bond formation. However, for the majority of an individual's life, these behaviors are almost exclusively used for pair-bond maintenance. The regulation of courtship and pair-bond formation has been studied in zebra finches (Arnold, 1975; Goodson and Adkins-Regan, 1999; Harding and Rowe, 2003; Tomaszycki and Adkins-Regan, 2005; Tomaszycki et al., 2006; Smiley et al., 2012), and there is evidence that affiliation associated with courtship behaviors (e.g., male song and sexual displays) is regulated by sex steroids (Arnold, 1975; Adkins-Regan and Leung, 2006; Harding and Rowe, 2003; Hill et al., 2005; Remage-Healey et al.,

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2008, 2009). However, the mechanisms and role of sex steroids in regulating behaviors associated with pair maintenance are largely unknown.

Sex steroids such as estradiol can regulate behavior by modulating gene transcription (i.e., "genomic" mechanisms) or by modulating intracellular signal transduction pathways (i.e., "non-genomic" mechanisms) (Balthazart et al., 2006). Note, however, that intracellular signal transduction pathways can affect gene transcription (Björnström and Sjöberg, 2005). Genomic effects of estradiol on behavior generally take hours to days; non-genomic effects of estradiol on behavior can occur within 15–30 min. In general, brain-synthesized estradiol appears more likely than gonad-synthesized estradiol to act via non-genomic mechanisms (Balthazart et al., 2004; Schmidt et al., 2008; London et al., 2009).

Brain-synthesized estradiol can rapidly increase aggressive and sexual behaviors in several species (Cross and Roselli, 1999; Balthazart et al., 2004; Cornil et al., 2006). Brain synthesis of steroids (neurosteroids) can explain how steroids regulate behaviors even when gonadal secretion of sex steroids is low. For example, in song sparrows (*Melospiza melodia*), aggressive behaviors are regulated by brain-synthesized estradiol in the non-breeding season, whereas they are regulated by gonad-synthesized sex steroids in the breeding season (Soma et al., 2000; Schmidt et al., 2008). Breeding readiness is not dichotomous for the opportunistically-breeding zebra finch, but rather it is a complex continuum that appears to be largely regulated by water availability, both in the wild and laboratory (Vleck and Priedkalns, 1985; Zann et al., 1995; Perfito et al., 2007). However, distinct endocrine states (brain and circulating steroid levels) are seen in breeding and non-breeding zebra finches (Prior et al., 2013). Furthermore, there may be an up-regulation of brain-synthesized estradiol and testosterone in behaviorally-relevant brain regions of non-breeding male and female zebra finches, consistent with the pattern seen in song sparrows (Prior et al., 2013). Taken together, these data raise the hypothesis that non-genomic steroid signaling mechanisms might be more important in the regulation of behavior in water-restricted zebra finches than breeding zebra finches.

Here, we examine the effects of acute (Experiment 1) and chronic (Experiment 2) aromatase inhibitor (fadrozole) treatment on the pair-maintenance behaviors of water-restricted (i.e., non-breeding) zebra finch pairs. If estradiol promotes pair-maintenance behaviors via non-genomic mechanisms, then fadrozole treatment would decrease these behaviors in both experiments. If estradiol promotes pair-maintenance behaviors via genomic mechanisms only, then fadrozole treatment would decrease these behaviors in Experiment 2 only. Alternatively, if these behaviors are not promoted by estradiol, then neither acute nor chronic fadrozole treatment would decrease these behaviors.

2. Materials and methods

2.1. Subjects

Subjects were adult (>120 d old) captive zebra finches housed in a colony maintained on a 14:10 h light:dark cycle. 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. Pairs were housed in cages ($38^{1/2} \times 19^{3/4} \times 19$ in, Corners Cages), in which a solid divider had been placed down the middle. Each pair therefore occupied half of the cage. Prior to the start of the study, pairs were provided a nestbox ($5^{1/2} \times 5^{1/2} \times 7^{1/2}$ in) and nesting materials. Pairs were housed together for a minimum of 2 months prior to the start of the experimental manipulation, and all pairs engaged in affiliative, courtship, and/or nesting behaviors, and were thus considered pair-bonded.

All subjects were water-restricted over the course of 4 weeks, from 6 mL of water to a minimum of 1 mL per pair per week (i.e., 3 mL to 0.5 mL per subject per week). Here, the water for a pair was always split between two water towers, to prevent one individual from monopolizing all of the water. This protocol for water restriction is intermediate between complete water deprivation (Sossinka, 1974) and more gradual water restriction over 11 weeks (Perfito et al., 2006). Additionally, this protocol is modified based on the amount of water consumed during the water restriction period of our previous study (1 mL per subject per week, Prior et al., 2013). Zebra finches are opportunistic breeders, and water restriction is highly effective at reducing breeding readiness. In females, reproductive organs (oviduct and ovary) are profoundly reduced by water restriction (Prior et al., 2013). Additionally, in males, circulating testosterone levels are significantly decreased (Prior et al., 2013). At the level of the pair, the number of eggs laid and the time spent engaging in breeding-related behaviors is decreased (Prior et al., 2013). In the current study we saw very few eggs laid, and male plasma testosterone levels were similar to water-restricted males in our previous study (Prior et al., 2013). For half of the pairs, the female was designated the focal subject, and for the other half of the pairs, the male was designated the focal subject.

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.

2.2. General timeline

A within-subjects design was used for the acute and chronic fadrozole (FAD) experiments (Experiment 1 and Experiment 2, respectively), and the same individual within a pair was the focal subject for both experiments. To minimize the stress of administration, fadrozole was delivered orally using a micropipette (Saldanha et al. 2004; Lee et al., 2007; Kabelik et al., 2011). The behavioral test was a Partner Separation and Reunion test (Fig. 1, see below). The focal subject received both the vehicle and fadrozole treatments for both experiments, and the behavior of each pair was assessed a total of four times. The order of treatment was counterbalanced within each experiment. There were washout periods between treatments within an experiment and also between the two experiments (Fig. 1A). In Experiment 1 (acute effects of fadrozole), the Partner Separation and Reunion test was administered immediately after the focal subject received fadrozole (Fig. 1A). In Experiment 2 (chronic effects of fadrozole), the focal subject received fadrozole daily for 1 week, and the following day (within 22 h of the last dosing), the Partner Separation and Reunion test was administered (Fig. 1A). In Experiment 2, immediately following the behavioral test, the focal subject was caught, and a blood sample was collected from the brachial vein and body mass was recorded. Blood samples were used for measurements of corticosterone, testosterone and estradiol.

We used a different dose of fadrozole for acute versus chronic administration. For Experiment 1, the focal subject received a single dose of 500 μg fadrozole in 20 μL of saline (~36 mg/kg) or vehicle orally via micropipette. This dose was tested in a pilot study (see below). For Experiment 2, the focal subject received daily doses of 300 μg fadrozole in 20 μL of apple juice (~21 mg/kg) or vehicle orally, every day for 7 d (between 14:30 and 18:30 h). In Experiment 2, apple juice was used as the vehicle to mask the taste of the fadrozole, which zebra finches appear to find unpalatable (our personal observations).

2.3. Pilot study: acute effects of fadrozole treatment

In a pilot study, we examined the rapid effects of orally administered fadrozole on estradiol and testosterone levels in plasma and