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Context-specific effects of estradiol on spatial learning and memory in the zebra finch

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ABSTRACT

Estradiol is known to impact cognitive function including spatial learning and memory, with studies focused largely on rodent models. Estrogens can be produced peripherally or centrally as neuroestrogens, and the specific role for neuroestrogens in memory processes remains unresolved. Many songbirds possess remarkable spatial memory capabilities and also express the estrogen synthetic enzyme aromatase abundantly in the hippocampus, suggesting that locally-produced estrogens may promote the acquisition or retrieval of spatial memories in these birds. We examined the effect of estradiol on spatial memory in three contexts in the zebra finch: retrieval after discrimination training, retrieval after familiarization but without discrimination training, and memory acquisition, using a combination of estradiol implants and oral dosing with the aromatase inhibitor fadrozole (FAD). Retrieval of spatial memory in both contexts was impaired when estradiol production was blocked. However, spatial memory acquisition was enhanced when estradiol production was inhibited whereas estradiol replacement impaired acquisition. These results provide evidence for a context-specific role of estradiol in songbird spatial memory, results that find accord with some mammalian studies but have not yet been observed in birds.

1. Introduction

Steroid hormones regulate numerous physiological and behavioral systems, including the neural circuits associated with spatial learning and memory. Spatial memory is reliant on the hippocampus (HP), and both testosterone and estradiol can influence the acquisition, retention, and retrieval of spatial memories (Barha & Galea, 2010; Leonard & Winsauer, 2011; Luine, 1997, 2008). In mammals, these sex steroids stimulate memory acquisition and retrieval in part by increasing dendritic spine density (Luine, 1997; Phan et al., 2012) and synapse formation (Mendez, Garcia-Segura, & Muller, 2011). In songbirds, estradiol or testosterone implants can also stimulate spatial memory acquisition and increase the size of hippocampal cells, whereas dihydrotestosterone (DHT) has no effect (Oberlander, Schlinger, Clayton, & Saldanha, 2004). Presumably, testosterone is locally aromatized into estradiol to promote spatial memory acquisition as aromatase is expressed abundantly in the songbird HP (Saldanha, Popper, Micevych, & Schlinger, 1998; Saldanha et al., 2000; Shen, Schlinger, Campagnoni, & Arnold, 1995). Overall, however, studies examining the effect of estradiol on spatial memory acquisition and retrieval are often contradictory: some identify beneficial effects of estradiol on all aspects of spatial memory, while others indicate that estradiol impairs spatial memory acquisition (e.g., Daniel, Fader, Spencer, & Dohanich, 1997; Gibbs, 2000; Snihur, Hampson, & Cain, 2008).

Many species of birds exhibit exceptional spatial memory capabilities, especially some food-storing oscine songbirds (e.g., Paridae and Corvidae; Bednekoff, Balda, Kamil, & Hile, 1997; Gould, Ort, & Kamil, 2012; Roth, LaDage, & Pravosudov, 2012; Salwiczek, Watanabe, & Clayton, 2010; Shettleworth, 1990), yet relatively few studies have investigated how sex steroids regulate avian spatial memory and cognition. Although the HP differs in structure and position between mammals and birds, it is analogous in function (Colombo & Broadbent, 2000). For example, lesioning of the songbird HP produces deficits in spatial memory (Bischof, Lieshoff, & Watanabe, 2006; Hampton & Shettleworth, 1996) which are reversed with fetal hippocampal implants (Patel, Clayton, & Krebs, 1997). In addition, expression of immediate early genes (c-fos and ZENK) is up-regulated in the zebra finch HP during spatial task learning and recall (Mayer, Watanabe, & Bischof, 2010). Problematically, of the few studies that have examined the effect of sex steroids on spatial memory in birds, testing methodologies, methods of hormone delivery, and subject sex have differed, making

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definitive conclusions hard to establish. For example, Oberlander et al. (2004) used subcutaneous implants that produced long-term changes in circulating hormone levels and assessed the effects on learning and memory for hidden food in male zebra finches (*Taeniopygia guttata*). In contrast, Hodgson, Meddle, Christians, Sperry, and Healy (2008) examined effects of orally delivered steroid hormones on spatial memory in a delayed non-matching to sample task in male and female great tits (*Parus major*). This allowed for manipulation of hormone levels on a finer scale, i.e., a day-to-day basis. Interestingly, while Oberlander found a strong effect of estradiol on spatial memory acquisition, the effect of estradiol on memory was less clear in Hodgson et al. Clearly additional studies are warranted to further our understanding of the complex relationship between steroid hormones and spatial memory.

The goal of the present study was to assess the role of estrogens in promoting spatial memory acquisition and retrieval in zebra finches, with an emphasis on understanding the context-specific effects of estradiol. To this end, birds were tested in a four-arm maze based on the similar food-finding task used by Bischof et al. (2006) that confirmed the role of the HP in spatial memory. We adapted the task to more closely resemble the radial-arm maze paradigm commonly used to test spatial memory in rodents (e.g., Olton, Collison, & Werz, 1977). A similar maze was previously used successfully to test zebra finch learning of a spatial memory task (Spence, Zhen, White, Schlinger, & Day, 2009).

Three experiments were conducted using a combination of estradiol implants and oral dosing with fadrozole (FAD), an effective inhibitor of the estrogen synthetic enzyme aromatase in songbirds (Wade, Schlinger, Hodges, & Arnold, 1994). In the first experiment, we allowed birds to learn the location of a food source (familiarization) and complete discrimination training for three days, then blocked estradiol synthesis and assessed the impact on memory retrieval. In experiment two, we examined the effect of aromatase inhibition with or without estradiol replacement on spatial memory retrieval after learning the location of the food source during the familiarization period but without discrimination training. Discrimination training was excluded in this experiment to limit the reinforcement created by multiple days of practice. In the final experiment, we examined the effects of aromatase inhibition with or without estradiol replacement on memory acquisition.

2. Materials and methods

2.1. Subjects

This study used non-breeding female zebra finches of breeding age (>100 days of age) that were communally housed in our zebra finch colony at the University of California, Los Angeles. Prior to being used in experiments, all birds were maintained on a 14:10 light: dark cycle and provided with *ad libitum* water, grit, cuttlebone, and seed, supplemented with egg mix, spinach, and bird vitamins. All experimental procedures were approved by the University Chancellor's Animal Research Committee.

2.2. Behavioral test

The apparatus was a four-arm maze similar to that used in Spence et al., 2009 (length = 44.8 cm; width of each arm = 13.0 cm; height = 45.7 cm). The maze sat on the floor of an 125 by 134 in. testing room, and was constructed with Plexiglas or wooden supports surrounded by screening (1 × 1 mm mesh) on all sides, giving the birds free visual exposure to the complex features of the testing room. Each arm provided a different view of the room: arm 1 provided a view of the testing chair, partition, and support beam, arm 2 a view of the back wall and side view of the counter and sink, arm 3 a straight-on view of the counter and sink, and arm 4 a view of a wall and side view of the support beam (Fig. 1). For each bird, one arm of the maze was randomly selected as the reward arm. The reward arm contained a red food cup from which the bird could feed. Identical red plastic food cups were placed in two of the remaining three arms, but the food in these cups was covered with a thin sheet of clear plastic to prevent access ('dummy cups'). On the morning of each testing day, the bird was food deprived for 3–4 h while water remained in the maze. Testing consisted of three 30-min sessions per day. The bird was allowed to complete as many trials as possible over the course of each session. Sessions were separated by approximately 1–2 h to enable synaptic consolidation, the first step in long-term memory formation (Dudai, 2004). During this period the bird remained in the maze with water, all food cups were removed, and the observer was absent from the room. To initiate testing, the bird was placed in the fourth arm of the maze in the dark; the lights were turned on, and the bird was allowed to search for the rewarded food cup and feed for several seconds (equal to one trial; max feeding time of 10 s). Afterwards, the lights were turned off and the bird's position was randomly changed to one of the other three arms; a dummy cup was placed in the newly emptied arm. In this way, the bird's starting location changed for each trial, while the location of the rewarded food cup remained constant. If a bird failed to complete a trial within 10-min, the light was turned off and a new trial was initiated. We recorded the number of errors made in each trial, with an error being defined as entry into an incorrect arm, even if the bird did not reach the food cup at the end of the arm. In addition, we recorded the amount of time each bird took to find the correct food cup per trial in experiments 2 and 3 (see below). Different birds were used for each of the three experiments described below.

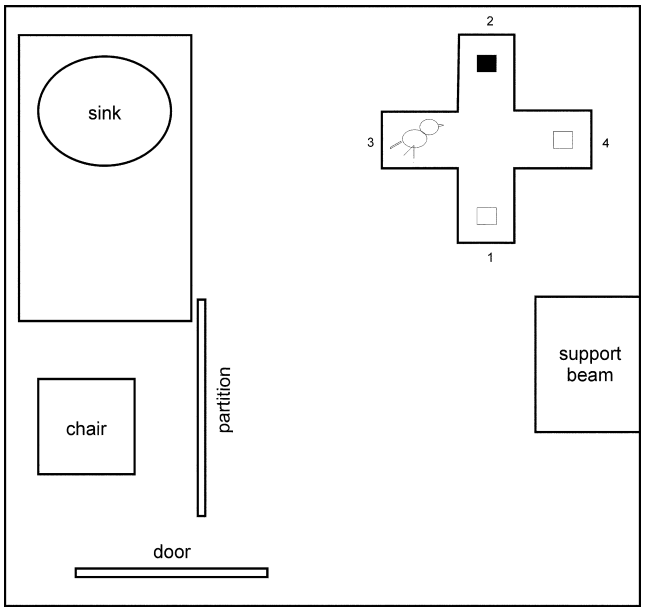

Fig. 1. Layout of the experimental testing room. The maze sat in one corner of the room opposite of the observer's chair and partition. External cues include a chair, partition and sink, as well as other cues such as a garbage can and light fixtures. In this example, arm 2 is the rewarded arm (black rectangle represents food cup), while arms 1 and 4 contain dummy food cups. The bird therefore starts the trial in arm 3. After completion of the trial, the bird is moved to arm 1, 3, or 4 (randomly determined), while the rewarded arm stays constant.