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Effects of housing condition and early corticosterone treatment on learned features of song in adult male zebra finches

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ABSTRACT

Early developmental stress can have long-term physiological and behavioral effects on an animal. Developmental stress and early corticosterone (Cort) exposure affect song quality in many songbirds. Early housing condition can act as a stressor and affect the growth of nestlings and adult song, and improvements in housing condition can reverse adverse effects of early stress exposure in rodents. However, little is known about this effect in songbirds. Therefore, we took a novel approach to investigate if housing condition can modify the effects of early Cort exposure on adult song in male zebra finches. We manipulated early housing conditions to include breeding in large communal flight cages (FC; standard housing condition; with mixed-sex and mix-aged birds) versus individual breeding cages (IBC, one male–female pair with small, IBC-S, or large clutches, IBC-L) in post-hatch Cort treated male birds. We found that Cort treated birds from IBC-S have higher overall song learning scores (between tutor and pupil) than from FC but there is no difference between these groups in the No-Cort treated birds. When examining the effects of Cort within each housing condition, overall song learning scores decreased in Cort treated birds from flight cages but increased in birds from IBC-S compared to controls. Likewise, the total number of syllables and syllable types increased significantly in Cort treated birds from IBC-S, but decreased in FC-reared birds though this effect was not statistically significant. These findings suggest that the effects of early Cort treatment on learned features of song depend on housing condition.

Introduction

Experiencing early developmental stress can have long-lasting effects on the physiology, behavior, reproductive success and survival of an individual (Lindstrom, 1999; Naguib and Nemitz, 2007; Naguib et al., 2006). Most songbirds learn songs from conspecific tutors (Clayton, 1987) and in many songbirds including zebra finches, song is important for mate choice. Songbird females typically prefer males that spend more time singing and this correlates with other fitness benefits such as territory quality, food availability and increased parental care (Alatalo et al., 1990; Greig-Smith, 1982). Furthermore, females of many songbird species prefer males with a higher song output (e.g. singing time, singing rate, and song amplitude) or males with more complex songs (measured as song duration or repertoire size; reviewed by Catchpole and Slater, 2008; Searcy and Yasukawa, 1996). In zebra finches, females develop a preference for songs similar to the songs of their fathers over those of conspecifics (Riebel et al., 2002; Riebel, 2000) as well as preferring males with higher song rates (Zann, 1996). Male zebra finches learn their song from their father (tutor) during a critical developmental period and the songs of adult bird include a series of introductory notes followed by a stereotyped sequence of identical song motifs consisting of several distinct song syllables (Sossinka and Bohner, 1980; Zann, 1993).

According to the developmental stress hypothesis, stress in early life affects brain development as well as the learning and production of song resulting in long-term effects on song (MacDonald et al., 2006; reviewed by MacDougall-Shackleton and Spencer, 2012; Nowicki et al., 1998, 2002; Spencer et al., 2003), suggesting a direct link between brain and behavior.

In songbirds, studies indicate that early life stress affects features of learned song. For example, in swamp sparrows, early food restriction results in less accurately copied song and an extended period of subsong (Nowicki et al., 2002). In zebra finches, the effects of early development stress, including food restriction (Brumm et al., 2009; Buchanan et al., 2004; Spencer et al., 2003; Zann and Cash, 2008), glucocorticoid hormone treatment (e.g. Cort; Buchanan et al., 2004; Spencer et al., 2003), and brood size manipulation (de Kogel and Prijs, 1996; Gil et al., 2006; Holveck et al., 2008; Naguib et al., 2006, 2008; Soma et al., 2006; Tschirren et al., 2009) on song quality of adult males have been investigated and there are some inconsistencies regarding which song parameters are affected by exposure to early developmental stress.

In zebra finches both early food restriction and early Cort treatment altered various song parameters. Early food restriction resulted in reduced song duration (Spencer et al., 2003), reduced syllables per song

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(Buchanan et al., 2004), reduced accuracy of song syntax learning (Brumm et al., 2009), slower syllable rate, increased song duration, and some effects on copying accuracy (Zann and Cash, 2008). Likewise, early Cort treatment resulted in reduced song duration (Spencer et al., 2003) and reduced song syllables per song (Buchanan et al., 2004).

Brood size manipulation in zebra finches resulted in a range of findings. Some studies indicate that this type of manipulation did not affect song accuracy or any song parameters although it affected physiological condition (Gil et al., 2006; Naguib et al., 2006, 2008). Two other studies demonstrated that brood size manipulation had opposite effects on song, for example, results from one study suggested that birds from larger broods had a reduced song rate (de Kogel and Prijs, 1996) whereas others have demonstrated that birds from larger broods had an increased song rate (Tschirren et al., 2009). Lastly, studies in Bengalese finches (Soma et al., 2006) and zebra finches (Holveck et al., 2008) indicate that brood size manipulation resulted in reduced syntactical copying. Stress levels from brood size manipulation depend on the housing conditions and timing of the manipulation. These reasons may explain a range of findings from brood size manipulation studies.

Environmental condition has an important role in regulation of brain, behavior, and physiology in biological, social, and medical sciences (Pryce et al., 2002). Animals (such as mice and rabbits) kept in enriched conditions show reduction of anxiety and fear (Chapillon et al., 1999; Hansen and Berthelsen, 2000), reduction of stress (Chamove, 1989; Van Loo et al., 2004), and better learning abilities (using the Hebb–Williams maze in female rats; Patterson-Kane et al., 1999) than those kept under standard housing conditions.

Research on rodents indicates that environmental enrichment can alter the effects of early stress exposure. Environmental enrichment (including daily handling, larger cages, larger number of animals per cage, more toys that were replaced regularly) in rats during the first two weeks of life reverses the effects of postnatal maternal separation on Hypothalamic–Pituitary–Adrenal (HPA) axis activation in adulthood and behavioral responses to stress (behavioral test of fearfulness: decreased exploration; Francis et al., 2002; reviewed by Nithianantharajah and Hannan, 2006).

Although little is known about the effects of environmental enrichment on early life stress in songbirds, previous studies indicate that large brood size can itself be a stressor possibly because of higher demands of more offspring (or increased competition between siblings) and less parental care due to the larger brood size, whereas, small brood size can be considered as an improved environment because of increased parental care and less competition between siblings. One question that remains unanswered is whether improved (less crowded) rearing conditions can modify the effects of early stress exposure on learned song features in male zebra finches. Zebra finches (*Taenopygia guttata*) are powerful models for studying the effects of early developmental stress on brain and song behavior. Therefore, we took a novel approach to examine if housing conditions (with varying degrees of crowdedness and brood size) can alter the effects of early Cort exposure on learned song features of adult male zebra finches. This was accomplished by manipulating early housing conditions, breeding in large communal flight cages (standard condition) versus individual breeding cages with small clutches (improved condition) or large clutches (poor condition). For early stress exposure, post-hatch male zebra finches received Cort implants since early Cort treatment mimics the effects of natural stressor such as food restriction on song quality and song learning accuracy (Buchanan et al., 2004; Spencer et al., 2003). After Cort implantation, zebra finches were reared under these three housing conditions.

Materials and methods

Animals

Male zebra finches from our breeding colony at Georgia State University (GSU) were used for this study. The GSU Institutional Use and Animal Care Committee granted approval for all animal procedures. Male birds age P4 ± 1 (4 days post-hatch; P3–P5) and adult (>90) were used. The sex of the birds was determined via PCR following the protocol of Soderstrom et al. (2007). Subjects were housed in nest boxes (with nesting material) in one of the two housing conditions, individual breeding cages (IBC; 16″L × 22″W × 16″H) with their parents and nestlings, or in large flight cages (FC; 60″L × 30″W × 72.5″H) with multiple families and mixed age cohorts. Air temperature was held constant at 20–22 °C and relative humidity maintained at 30–50%. The light cycle was 12:12 h light:dark. The cages were sprayed with water once a day every day to imitate rainfall and facilitate breeding because as opportunistic breeders zebra finches nest during rainy periods. The birds were fed an *ad libitum* diet of standard finch millet seed. In addition, they were given a mixture of fresh greens (spinach) and a protein supplement (boiled eggs) once a week. Fresh drinking water (changed daily), oyster shell grit and cuttlebone were provided.

Cort implants were made of Dow Corning silastic tubing (0.76 mm ID × 1.19 mm OD) in two lengths (5 mm and 10 mm) and packed with crystalline Cort (catalog C2505-500 MG, Sigma-Aldrich, St. Louis, MO) and sealed at the two ends with silicone paste and implanted subcutaneously over the pectoral flight muscle. The effective lengths of 10–15 mm have been used in adult songbirds (such as free-living sparrows) to elevate Cort concentration moderately above baseline level (Astheimer et al., 2000; Breuner and Hahn, 2003; Martin et al., 2005; Newman et al., 2010). We used an effective length of 10 mm for adult zebra finches and 5 mm for P4 ± 1 zebra finches. In previous studies, Cort release from silastic implants was facilitated by making a small hole in the implant (Astheimer et al., 2000; Newman et al., 2010; Silverin, 1998). Using a 26-gauge needle, a single hole was made near the end of the tubing (but not through the silicone plug) in each Cort implant used for P4 ± 1 and adult birds.

Validation of Cort implants in vivo

To validate Cort implants *in vivo* adult male zebra finches were implanted subcutaneously above the pectoral flight muscle with either 10 mm Cort ($n = 5$) or 10 mm empty ($n = 4$) implants. Two days before implantation (day −2), baseline blood samples were collected. Blood samples were also collected on day 1 (one day after implantation), day 3, and day 7. Blood samples were collected by venipuncture of the alar wing vein using a 26-gauge needle and blood was then collected into heparinized microhematocrit tubes. Blood collection occurred within 3 min after capture at the same time each day (around 11 am) for each bird. Rapid blood collection is important to confirm that the Cort levels measured here resulted from the treatment and not from capture stress. Blood samples were kept on ice until centrifuged. Then, after centrifuging blood, plasma was collected and stored at −20 °C until analysis. One of the birds from the Cort treated group died on day 3 after implantation and these samples were not used in the analysis.

Hormone assays

Total plasma Cort concentrations were measured in adult birds using a Corticosterone RIA kit (Corticosterone ^{125}I RIA kit, catalog # 07-120102; MP Biomedicals LLC, Solon, OH, for rat plasma). We validated the use of this kit for measuring plasma Cort concentration in zebra finches, and this kit has been used in previous studies in song sparrows as well (Newman et al., 2010). To validate the assay, we initially ran zebra finch pooled plasma with rat and hamster pooled plasma. The standard curve was matched to the zebra finch Cort concentrations range (by adding 6.25 ng and 12.5 ng through serial dilution of 25 ng standard to increase sensitivity). Once this was established, we ran a dilution series (1:2 98%, 1:4 102%, and 1:6 115%) of zebra finch plasma to get linearity and obtained the overall recovery. In addition to the two (low and high concentrations of Cort) controls that were provided by