Corticosterone exposure during development improves performance on a novel foraging task in zebra finches

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Developmental stress affects a range of phenotypic traits in later life-history stages. These long-term effects are thought to provide information to potential mates on individual quality and the ability to cope with adversity (i.e. the developmental stress hypothesis). Developmental stress appears to affect learning broadly, but the direction of effect is not always consistent between studies. This disparity may arise from indirect effects of developmental stress on other physiological or behavioural systems, which can affect outcomes in learning paradigms. Here, we examine the effect of elevated corticosterone (the dominant avian stress hormone) during development on the ability of zebra finches, *Taeniopygia guttata*, to learn a novel foraging task as adults. Additionally, we evaluate treatment effects on metabolism, to determine whether changes in metabolic rate indirectly alter learning results. We found that birds exposed to elevated corticosterone during development solved a foraging task faster than control siblings. This outcome could result from differences, not in learning ability, but in motivation for reward. However, we found no difference between treatment groups in metabolic rate. Hence, our results indicate that developmental stress can increase learning ability, and suggest that the effects of stress will vary based on the type of learning studied.

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Stress during development can affect a range of physiological and behavioural systems resulting in outcomes such as reduced growth, impaired immunocompetence and altered neurological function (e.g. Liu et al., 1997; Loiseau, Sorci, Dano, & Chastel, 2008; Müller, Jenni-Eiermann, & Jenni, 2009; Weaver et al., 2004). Phenotypic effects shaped by developmental stress can be sustained across an animal's lifetime and, in this way, may have important effects on fitness across life-history stages (reviewed in: Matthews, 2005; Nesan & Vijayan, 2005; Schoech, Rensel, & Heiss, 2011; Spencer & MacDougall-Shackleton, 2011). For example, songbirds learn their species-specific song early in life (Beecher & Brenowitz, 2005; Brenowitz & Beecher, 2005; Marler, 1970). Developmental stress (e.g. food restriction or elevated glucocorticoid stress hormones) decreases development of the brain regions that control song learning and production (Buchanan, Leitner, Spencer, Goldsmith, & Catchpole, 2004; Nowicki, Searcy, & Peters, 2002). Adults exposed to stress during development sing less complex songs and are, consequently, less preferred by females (Buchanan, Spencer, Goldsmith, & Catchpole, 2003; Nowicki et al., 200; Spencer, Buchanan, Goldsmith, & Catchpole, 2003; Spencer et al., 2005). In this way, adult song signals an individual's ability to cope with an adverse environment during development and is a reliable signal for mate choice (i.e. the developmental stress hypothesis; Nowicki, Peters, & Podos, 1998; Nowicki et al., 2002; Spencer et al., 2003).

Over the years, substantial evidence from studies in both free-living and captive birds has supported the developmental stress hypothesis (reviewed in Spencer & MacDougall-Shackleton, 2011). Recently, the evaluation of this hypothesis has been expanded to examine how developmental conditions affect learning tasks other than song learning. For example, Bonaparte, Riffle-Yokoi, & Burley (2011) restricted the protein content of food for developing zebra finches, *Taeniopygia guttata*, and found that food-restricted birds had reduced ability to solve an associative-learning task as adults (175 days posthatch). Black-legged kittiwakes, *Rissa tridactyla*, chicks exposed to experimentally elevated levels of corticosterone (CORT; the dominant avian glucocorticoid) had a reduced ability to complete an associative-learning task as juveniles and continued to perform poorly 8 months later as adults (Kitaysky, Kitaiskaia, & Winfield, 2003). Other studies have found a positive effect of

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developmental stress on learning. Domesticated chickens, *Gallus gallus domesticus*, subjected to social stress during the first 3 weeks of life performed better at an associative-learning task compared to control birds (Goerlich, Natt, Elfwing, & Macdonald, 2012). Likewise, juvenile Japanese quail, *Coturnix japonica*, exposed to repeated negative stimuli (i.e. stress) displayed enhanced behavioural flexibility in a spatial memory task (Calandreau et al., 2011). In summary, developmental stress has broad effects on learning, but the direction of effect is not consistent between studies.

Developmental stress is known to affect a range of phenotypic traits that could indirectly influence learning and potentially explain variable results between studies. For example, in zebra finches, treatment with CORT during the nestling period increased variability in overnight standard metabolic rate (Spencer & Verhulst, 2008). However, this effect was only observed during the treatment period and not in adulthood (Spencer & Verhulst, 2008). In contrast, Schmidt, MacDougall-Shackleton, and MacDougall-Shackleton (2012) showed that developmental stress in the form of food restriction and elevated CORT permanently increases standard metabolic rate in female song sparrows, *Melospiza melodia*. Birds with greater metabolic demands could be differentially motivated by food reward in learning paradigms and, thus, solve paradigms faster independent of learning ability. Developmental stress can also affect activity level and behaviours such as neophobia, which could confound the results of experiments that measure learning using novel objects. Studies incorporating additional metrics of adult phenotype would be better able to elucidate the relationship between development stress and learning.

We examined the effects of elevated CORT during the nestling period on adult learning in zebra finches. We fed zebra finches CORT during the nestling period and measured learning in adult birds (60 days posthatch) using a foraging paradigm that quantifies the ability of birds to access a hidden seed reward (Boogert, Giraldeau, & Lefebvre, 2008; Grindstaff, Hunsaker, & Cox, 2012). Based on the developmental stress hypothesis, we predicted that zebra finches fed CORT during development would solve the learning task more slowly than control birds. Developmental stress can also affect metabolic rate in birds (e.g. Schmidt et al., 2012; Spencer & Verhulst, 2007). Differences in metabolic rate between CORT-fed and control birds could increase energetic needs, resulting in greater motivation to feed and, hence, locate food. Thus, we also tested whether CORT treatment during development affected metabolic rate, which could help explain variation in performance on food-based learning tests.

METHODS

Study Population

We obtained adult domesticated zebra finches from six pet stores across Montana and Washington, U.S.A. We banded the birds with a unique combination of colour bands to identify individual birds. Breeding finches were housed in a 6.1×7.6 m room where they were allowed to interact freely with all other birds. We housed the birds on a 14:10 h light/dark cycle at 26–27 °C with 20–30% humidity. Birds had access to 12 nestboxes and shredded burlap nesting material. We fed birds commercial finch seed (Silver Song West) and spray millet ad libitum and supplemented their diet daily with hardboiled eggs, spinach and crushed eggshells. Nestboxes were monitored daily for signs of nest building and egg laying.

Starting on hatch day, we marked nestlings with an individual combination of leg markings using a black Sharpie marker. Between 3 and 4 days after hatching, we banded nestlings with a numbered plastic leg band. All nestlings in a nest were then randomly assigned to treatment groups (CORT or control). Nestlings exposed to the CORT treatment were fed oral boluses (25 μl) of CORT (Sigma Aldrich) dissolved in peanut oil twice daily approximately 5 ± 1 h apart. From 12 to 15 days posthatch, nestlings received 0.124 mg/ml of CORT in peanut oil for a total daily dose of 6.2 μg of CORT. Starting 16 days posthatch, the dose was increased to 0.163 mg/ml for a total daily exposure of 8.15 μg of CORT. Control nestlings were fed 25 μl of peanut oil on an identical feeding schedule. Nestlings were exposed to treatments from 12 to 28 days posthatch (methods as per Spencer, Evan, & Monaghan, 2009). At 30 days posthatch, zebra finches exposed to CORT treatment had elevated baseline and stress-induced CORT compared to control siblings (mean baseline: CORT: 2.41; control: 1.06 ng/ml; mean stress-induced: CORT: 8.26; control 4.58 ng/ml; Crino, Driscoll, & Breuner, 2014). However, there were no treatment effects on baseline or stress-induced CORT in zebra finches at 60 or 90 days posthatch (Crino et al., 2014).

We noticed no adverse effects from CORT treatment on the health or behaviour of nestlings. Our methods were approved by the Institutional Animal Care and Use Committee of the University of Montana (protocol number AUP 018-11).

Learning Paradigm

We measured learning ability in zebra finches 60 days posthatch (±2 days) using a foraging paradigm with four levels of escalating difficulty (methods as per Boogert et al., 2008). We presented birds with a plastic grid ($26 \times 22 \times 2$ cm) containing 10 wells (0.8 cm deep and 1.3 cm wide) covered with lids fitted with rubber bumpers on the bottom (3.5 cm in diameter). For each level, we placed two seeds of millet in every well of the testing apparatus. To proceed to the next level of difficulty in the paradigm, birds had to access and eat the seeds from at least two wells. For the first level of difficulty, we placed the lids next to the holes. For the second level, we placed the lids so they covered half of each well. For the third level, we covered the wells with the lids entirely, and for the fourth level, we pushed the rubber bumpers into the wells. To pass the fourth level of difficulty, birds had to pry the lids off with their beaks to access the seeds. Birds that solved the task in the fewest trials were considered superior learners (Boogert et al., 2008).

We isolated test birds in wire cages ($33 \times 38 \times 43$ cm) 24 h preceding the learning test in a room separate from the main colony. To prevent the birds from seeing each other during the test we placed opaque barriers between the cages. We removed all food from cages 1 h before the lights were turned off in the testing rooms at night, and we began the learning trials 1 h after the lights were turned on for the day. This protocol allowed us to standardize the fasting time and control for potential differences among individuals in motivation to solve the foraging paradigm. Birds were housed on a 14:10 h light/dark cycle and so were food deprived for 10 h at night and 2 h during the day. We observed no adverse effects of food deprivation on the health or behaviour of the birds. Throughout the course of the experiment birds had access to water ad libitum. We started the learning trials at 0730 hours. Learning trials were recorded using Veho Muvi microDV camcorders. An observer started the camera at the beginning of each trial before leaving the testing room. For each learning trial, birds had 15 min to solve the task and pass to the next stage. After each learning trial, we removed the lids from the apparatus to allow the birds access to the seeds in the open wells for 45 min before starting the next trial, to provide adequate nutrition during the experiment. Birds that failed to pass a stage were exposed to the previous stage in the next trial. For example, if a bird failed to pass stage 3, it was presented