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Long-term effects of adolescent stress on neophobic behaviors in zebra finches are modulated by social context when in adulthood

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ABSTRACT

Experiencing stress during adolescence can increase neophobic behaviors in adulthood, but most tests have been conducted in the absence of conspecifics. Conspecifics can modulate responses to stressors, for example by acting as 'social buffers' to attenuate the aversive appraisal of stressors. Here, we investigate the long-term effects of adolescent stress on the behavioral responses to novel stimuli (a mild stressor) across social contexts in an affiliative passerine bird, the zebra finch. During early (days 40–60) or late (days 65–85) adolescence the birds (n = 66) were dosed with either saline or the hormone corticosterone (CORT). CORT was given in order to mimic a physiological stress response and saline was given as a control. In adulthood, the birds' behavioral responses to a novel environment were recorded in both the presence and absence of conspecifics. An acute CORT response was also quantified in adolescence and adulthood. Our findings show clear evidence of social context mediating any long-term effects of adolescent stress. In the presence of familiar conspecifics no treatment effects were detected. Individually, birds dosed with CORT in early adolescence were slower to enter a novel environment, spent more time perching in the same novel environment, and, if female, engaged in more risk assessment. Birds dosed in late adolescence were unaffected. No treatment effects were detected on CORT, but adolescents had a higher CORT concentration than adults. Our results are the first to suggest that familiar conspecifics in adulthood can buffer the long-term effects of stress that occurred during early adolescence.

1. Introduction

Stimuli that are perceived as a (potential) threat activate the hypothalamic-pituitary-adrenal axis, a neuroendocrine cascade regulating the secretion of glucocorticoids (GCs), such as corticosterone (CORT) in rodents and birds (Romero, 2004; Sapolsky et al., 2000). GCs alter many physiological systems in order to facilitate an acute 'stress response' (McEwen and Wingfield, 2010; Sapolsky et al., 2000). The behavioral consequences of a stress response include increased locomotor activity, risk assessment, and risk avoidance (Haller et al., 1998; McNaughton and Corr, 2004; Rodgers et al., 1999), but increased risk-taking has also been noted (Martins et al., 2007; Toledo-Rodriguez and Sandi, 2011). Exposure to stressors during early post-natal development can have sustained effects on an adult's stress response (Sachser et al., 2011; Lupien et al., 2009; Monaghan, 2008), with adult neophobic behaviors and stressor-induced CORT concentration higher in those animals exposed to developmental stress compared to control animals (Hollis et al., 2013). GCs appear to be the endocrine mechanism behind the long-term effects of developmental stress, as direct elevation of CORT during development induces long-term effects on both neophobic behavior and stressor-induced CORT secretion in a number of bird species (Schoech et al., 2012), including zebra finches (*Taeniopygia guttata*; Spencer and Verhulst, 2007; Spencer et al., 2009). CORT likely has sustained effects on adult stress responses by altering neural expression of GC receptors, subsequently heightening the concentration of CORT secreted in response to stressors and in turn heightening the expression of neophobic behavior (Banerjee et al., 2012; Isgor et al., 2004; Zimmer and Spencer, 2014).

Adult social interactions have influence over responses to stressors, with conspecific presence attenuating or 'buffering' aversive responses to novelty and other stressors in several species of rodents (Beery and Kaufer, 2015; DeVries et al., 2003; Hennessey et al., 2009; Kikusui et al., 2006) and primates (Gunnar and Hostinar, 2015; Sanchez et al., 2015). In birds, familiar conspecifics (vs. single housing) also appear to act as social buffers noted by reduced latencies to feed from a feeder in the presence of a novel object in zebra finches (Coleman and Mellgren, 1994) and budgerigars (*Melopsittacus undulatus*; Soma and Hasegawa, 2004). However, European starlings (*Sturnus vulgaris*) do not appear to act as social buffers to one another (Apfelbeck and Raess, 2008). Absence of a social buffering effect in starlings likely reflects a floor effect, as so few birds engaged in the

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task of foraging from a familiar feeder after a novel object was attached to the feeder (Apfelbeck and Raess, 2008). Familiar conspecifics therefore tend to attenuate neophobic behaviors in adulthood, but no work has investigated whether familiar conspecifics can attenuate neophobic behaviors that were heightened in response to developmental stress.

Adolescence is a developmental stage that begins with puberty and ends with sexual maturity (Brown and Spencer, 2013; Spear, 2000). During adolescence, animals leave the natal home and become exposed to stressors, such as unfamiliar environments and predators (Spear, 2000; Yoder, Marschall & Swanson, 2004). Stressor exposure during adolescence can have long-term effects on an adult's stress response (Eiland and Romeo, 2013). Animals exposed to stressors during adolescence, compared to controls, display more neophobic behaviors in adulthood (Hollis et al., 2013) and have a higher CORT concentration in response to a stressor in adulthood (Isgor et al., 2004; Pohl et al., 2007). The neural development of HPA axis regulators, such as the amygdala, continues to occur during adolescence; with more pronounced changes occurring earlier in adolescence (Andersen and Teicher, 2008). Adolescents are also sensitive to steroid hormones, with neural sensitivity to steroid hormones declining with increasing age (Schulz et al., 2009). Resultantly, exposure to stressors during adolescence appears to have more pronounced effects on later-life responses to stressors the earlier in adolescence the developmental stress occurs (Tsoory and Richter-Levin, 2006). However, little work has attempted to investigate the importance of age-related changes in hormone sensitivity on the formation of adult phenotypes. In addition, the long-term effects of adolescent stress have only been quantified in an individual context. Whether familiar conspecifics in adulthood can buffer the long-term effects of adolescent stress on adult neophobic behavior and the stressor-induced secretion of CORT remain to be tested.

In the current study we investigated the long-term effects of adolescent stress on adult responses to novel stimuli, both individually and in the presence of familiar conspecifics, and the CORT response to a capture/restraint stressor. We used a highly social passerine bird, the zebra finch, expected to be sensitive to changes in social context. Zebra finches begin puberty around postnatal day 30 and reach sexual maturity by postnatal day 100 (Zann, 1996). Birds were orally dosed with CORT during adolescence in order to mimic a physiological response to a stressor. Birds were dosed either in early adolescence (postnatal days 40–60) or in late adolescence (postnatal days 65–85). Two treatment periods were chosen in order to investigate whether the effects of adolescent stress decline with age. The specific ages were chosen as they are ten days either side of behavioral changes that occur during adolescence in zebra finches. The birds begin to spend more with unfamiliar conspecifics by postnatal day 50 (Adkins-Regan and Leung, 2006) and begin to display sexual behaviors by postnatal day 75 (Zann, 1996). In adulthood, behavioral responses to a novel environment (avoidance, risk assessment, locomotor activity) were quantified after the birds had been single housed (individual context) and when the birds were in their home cages with their familiar cage mates (group context). CORT secretion in response to a capture/restraint stressor was also quantified, first in adolescence shortly after the treatment period ended and then in adulthood after behavioral testing was completed. We predicted that (1) bird's treated with CORT in adolescence would display more avoidant, locomotor, and risk assessment behaviors in an individual context novel environment in adulthood compared to saline treated birds, (2) no effects of adolescent CORT would be detected in a group context novel environment, (3) the secretion of CORT in response to capture/restraint would be higher in birds treated with CORT during adolescence compared to saline treated birds, and (4) birds treated with CORT in early adolescence would display more avoidant, locomotor, and risk assessment behaviors and have a higher CORT response than birds treated with CORT in late adolescence.

2. Methods

2.1. Experimental design

All birds were housed in a single room with a 12:12 light-dark cycle (lights on at 07:00), temperature at 22 ± 2°, and relative humidity at 55 ± 5%. All appropriate ethical guidelines and requirements were adhered to, as set out in the Principles of Laboratory Animal Care (NIH, Publication No. 85–23, revised 1985) and the UK Home Office Animals (Scientific Procedures) Act 1986 under project licence 70/8159 and personal licences IDFA58352, IEBE43CFF, and 60/13261.

Adult zebra finches (n = 20 male, 20 female) from an in-house breeding stock were housed in one of two 100 cm long × 100 cm high × 50 cm deep white wrought iron cages (10M, 10F per cage) and observed for two one hour sessions per day (one 8:30–13:00, one 13:00–17:30) for 14 days. Birds had ad libitum access to seed (Food for Finches, Johnson & Jeff, UK) hoppers, water hoppers, and water baths. Spinach was provided on days one, six, and eleven. When two birds engaged in any mating behavior (following, directed song, mounting) on three consecutive sessions the pair were removed from the cage and housed together. Each pair (n = 20) was housed in one half of a breeding cage (MB 3612 Metal Double Breeding Cage, R.J. Leigh Ltd., UK) with each cage half measuring 60 cm × 50 cm × 50 cm (length × height × depth). Each cage half was separated by a solid metal divider. The cage bases were covered with wood pellets (Stovies Wood Pellets, Arbuthnott Wood Pellets Ltd., UK) and contained two plastic perches. Birds had ad libitum access to seed and water hoppers, a grit tray, and a water bath like that for the partner choice cages.

A cardboard nest box was attached to the outside of the cage with an entrance facing the cage interior. Hay and jute fibre nesting materials were given daily until the beginning of incubation. Nests were inspected daily. Egg food (CéDé Premium Egg Food, Belgium) was provided daily until chicks reached nutritional independence. Hatch order can have long-term effects on adult exploratory behavior (Mainwaring and Hartley, 2013), so an attempt was made to synchronise hatching. Eggs were removed on the day they were laid and replaced with a fake 'Fimo' egg (Staedtler Fimo Soft Oven Hardened Modelling Clay (white), UK). After two consecutive days of not laying, eggs were returned to the nest to allow incubation. Eggs were candled at day seven, with any infertile clutches removed to allow relaying. On post-hatch day (PHD) 5, the chicks were given a temporary ID (coloured nail polish applied to each leg, re-applied on PHD 8) before being given a permanent ID (ne uniquely numbered orange leg ring and one coloured leg ring (pink, yellow, light blue, or white) on PHD 10. A blood sample (approximately 5 μl) was taken from the brachial vein of all chicks on one day between PHD 12–15 for molecular sexing (see below). After nutritional independence was achieved (day 35), the offspring (n = 66) were separated from their parents and re-housed in same-sex, non-sibling triplets in cages identical to the home cage (i.e. half of a full breeding cage with a solid divider separating the two halves and therefore two triplets). Triplets were matched for age (±2 days) and mass (within 1 g). The birds remained in these triplets until the end of the experiment. Each triplet was randomly distributed across four treatments: early adolescent CORT (E-CORT; n = 9M, 9F), early adolescent saline controls (E-SAL; n = 9M, 6F), late adolescent CORT (L-CORT; n = 9M, 9F), and late adolescent saline controls (L-SAL; n = 9M, 6F).

2.2. Molecular sexing

DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen Ltd.) following the protocol for nucleated erythrocytes. Extracted DNA was used to perform PCR to amplify CHD gene fragments with the primer pair P2 (5′-TCTGCATCGCTAAATCCTTT-3′)/P17 (5′-GAAGA AAATAATTCCAGAAGTCCA-3′) that has previously been developed for sexing zebra finches (Arnold et al., 2003). All PCR reactions were