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Repeated exposure to stressful conditions can have beneficial effects on survival

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ABSTRACT

Repeated exposure to stressful circumstances is generally thought to be associated with increased pathology and reduced longevity. However, growing lines of evidence suggest that the effects of environmental stressors on survival and longevity depend on a multitude of factors and, under some circumstances, might be positive rather than negative. Here, using the zebra finch (*Taeniopygia guttata*), we show that repeated exposure to stressful conditions (i.e. unpredictable food availability), which induced no changes in body mass, was associated with a decrease in mortality rate and an increase in the age of death. As expected, the treated birds responded to the unpredictable food supply by increasing baseline glucocorticoid stress hormone secretion and there were no signs of habituation of this hormonal response to the treatment across time. Importantly, and consistent with previous literature, the magnitude of hormone increase induced by the treatment was significant, but relatively mild, since the baseline glucocorticoid concentrations in the treated birds were substantially lower than the peak levels that occur during an acute stress response in this species. Taken together, these data demonstrate that protracted exposure to relatively mild stressful circumstances can have beneficial lifespan effects.

1. Introduction

Protracted or repeated exposure to stressful stimuli, such as those experienced by individuals living in uncontrollable or highly unstable environments, can have wide ranging effects on animal physiology, but the extent to which these effects are adaptive is the subject of considerate debate (Boonstra, 2013). In vertebrates, one of the main systems mediating responses to stressful environmental conditions is the Hypothalamic–Pituitary–Adrenal axis (HPA axis), which regulates both basal production and transient surges of circulating glucocorticoid stress hormones. Transient increases in circulating glucocorticoids are a highly conserved component of the vertebrate stress response and play a key role in initiating an array of metabolic changes intended to mobilise energy, including hepatic gluconeogenesis and inhibition of glucose uptake by peripheral tissues (Sapolsky et al., 2000). These changes are thought to be vital for promoting short-term survival (Wingfield et al., 1998; Sapolsky, 2000). At the same time, dynamic changes in basal level of glucocorticoids, such as those occurring across differing seasons within the same year in a variety of free-living vertebrate species, are also thought to be critical for survival (Romero, 2002). Over a longer time scale, however, the repeated activation of the HPA axis may lead to a dysregulation and dysfunction of the stress axis (Sapolsky, 2000).

The predominant view is that repeated stress exposure, and the consequent prolonged elevation of glucocorticoid levels, is harmful since it can induce a large variety of downstream negative effects, including impairment of brain functioning and immune responses (Sapolsky, 1992; de Kloet et al., 2005). As a consequence, it is often predicted that living in chronically stressful environments should result in long-term adverse health effects, and, therefore, in reduced longevity (McEwen and Wingfield, 2003). Support for this idea comes primarily from the biomedical field, but as recently noted, it does not quite hold up in some well-studied ecological systems (Boonstra, 2013). For example, snowshoe hares (*Lepus americanus*) show evidence of chronic stress during the cyclic population declines associated with high predation risk (Sheriff et al., 2011), but during these periods they show no sign of increased pathology or relevant dysfunction of the stress axis (Cary and Keith, 1979). It has been argued that, at least in these populations, animal responses to protracted or repeated stress exposure represent an evolved strategy that enables individuals to respond in a manner that gives the best fitness outcome when stressful conditions prevail (Boonstra, 2013).

Experimental studies of the link between stress exposure and longevity have had conflicting results. For example, in birds, experimental elevation of glucocorticoids by twice daily oral dosing during the nestling period in zebra finches, which increases the strength of the acute stress response (Spencer et al., 2009), causes a marked reduction in

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adult longevity (Monaghan et al., 2012). In contrast, exposure to an unpredictable food supply in juvenile grey partridges, presumed to be associated with increased glucocorticoid levels, was found to improve survival when the animals were released in the wild at adulthood (Homberger et al., 2014). Such differences might depend on the type and degree of stress exposure (Costantini et al., 2010), the stage at which the stressful environment was experienced, and for how long the exposure occurred. In both of the aforementioned studies, the manipulations were carried out in young individuals and the stressful circumstances were imposed over a relatively short-time scale (2–3 weeks). We do not know if the same effects could occur with stress exposure in adulthood, and importantly, we do not know how survival might be altered when stress exposure is experienced over a much longer time period. Furthermore, the level and pattern of increase in glucocorticoids induced by the stressor may be very important (Costantini et al., 2010). In the zebra finch study by Spencer et al. (2009), the oral dosing with hormones caused a rapid rise in circulating levels similar to that induced by an acute stressor and the apparently permanent increase in stress reactivity means that these animals are then exposed to higher glucocorticoids whenever stressors are encountered. It is possible that the elevation of hormone levels induced by an unpredictable food supply, which generally elevate baseline glucocorticoids (e.g. Pravosudov et al., 2001; Jenni-Eiermann et al., 2008), is less severe.

In this study, we examined the effects of prolonged and repeated exposure to stressful conditions in adulthood on long term survival in the zebra finch (*Taeniopygia guttata*). The stress exposure that we used was random withdrawal of food, mimicking a natural environmental stressor in unpredictable environments. Previous studies in birds that have used similar intermittent food withdrawal protocols have shown that they simulate stressful conditions since they often elevate endogenous glucocorticoid levels within the natural range (e.g. Pravosudov et al., 2001; Jenni-Eiermann et al., 2008). We exposed young adult females to unpredictable food availability. We monitored survival in both the experimental and control birds kept on ad libitum food until they were 3 years of age, a time window within which survival differences have been measured in this species in other studies (Monaghan et al., 2012; Costantini et al., 2014).

2. Materials and methods

2.1. Experimental design

We used females produced from two breeding events in a stock population of zebra finches maintained at the University of Glasgow (replicate 1 produced: April–June 2011; replicate 2 produced: August–September 2011 — full details in Supplementary material). The experiment started when the birds were fully adult and age was 152 ± 15 days (average ± SD — all ages are estimated from recorded hatching date of the first chick hatched in each clutch). They were moved into treatment-specific cages (n = 7–10 per 120 × 50 × 50 cm cage) and randomly assigned to two experimental groups: unpredictable food (replicate 1 = 49, replicate 2 = 66) or control (replicate 1 = 49, replicate 2 = 64). Females that hatched in the same nest were counterbalanced between the two treatment groups, and family of origin was taken into account in the analyses. Before the treatment started (when the birds were on average 152 days old), there were no differences in body mass (measured to the nearest 0.01 g) or structural size (measured to the nearest 0.01 mm using a digital calliper — Dial Max, Wiha, Switzerland) between treatment groups (mean ± SEM — body mass: control: 17.01 g ± 0.18, unpredictable food: 16.84 g ± 0.16; tarsus length: control = 14.81 mm ± 0.06, unpredictable food: 14.77 mm ± 0.05 — full statistics in Tables S1 and S3, Supplementary material). Adult tarsus length was measured in 204 out of 228 birds (control = 103; unpredictable food = 101). Tarsus was always measured by one experimenter; within experimenter error was tested in a subset of 30 birds and measurement repeatability for tarsus was very high (repeatability coefficient = 0.95, $p < 0.0001$, Lessells and Boag, 1987). Photoperiod was 14 h:10 h light:dark cycle and temperature was maintained between 20 and 24 °C.

Females in the unpredictable food treatment were denied access to food for a continuous period of 4.9 h a day (i.e. approximately one third of the daylight hours), 4 days per week on a random schedule (full details in Supplementary material). For the remaining two thirds of the day they had ad libitum food. Birds in the unpredictable food treatment were always maintained on this regime other than when breeding (breeding events, n = 3 at 188 ± 13, 408 ± 12 days, and 653 ± 11 days of age — mean ± SD for all) when they received ad libitum food continuously for approximately 2 months. Birds in the control treatment were always provided with ad libitum food and experienced exactly the same breeding regime as the unpredictable food birds. The unpredictable food treatment employed here was not designed to induce caloric restriction since the treated females had 65% of daylight hours to replenish their daily energy requirements. Females were weighed at regular intervals during the experiment. The difference in the time available for feeding did not have a significant influence on body mass dynamics since (1) average body mass tended to increase over time in the birds in both treatments (descriptive statistics in Table S2, Supplementary material), and (2) there were no overall significant effects of the treatment on this variable (full details in Table S3, Supplementary material). There is a suggestion in the latter analysis of a slight reduction in body mass in the unpredictable-food birds compared with the controls at the 1 year sampling point, but this difference was very small (unpredictable food birds on average 3.98% lighter than controls), and there was no significant treatment difference at any other point. In a subset of birds (n = 21 from each experimental group in replicate 1 birds) we also measured fat scores one month after the treatment regime had started (age birds, mean ± SD: 188 ± 13 days) on a scale ranging from 0 (no fat) to 5 (furcula and abdomen bulging with fat) following Busse (1974). There was no significant effect of the treatment on this variable (Pearson Chi-square = 0.38, df = 2, $p = 0.83$). Our treatment, therefore, primarily altered the temporal predictability of food resources rather than the daily overall food intake, mimicking an environmental stressor experienced by animals living in highly unstable environments, such as those with frequent inclement weather conditions (Wingfield and Kitaysky, 2002).

We monitored the survival of the birds for the same time period in the two replicates, three years (i.e. 1096 days of age). All procedures were carried out under Home Office Project Licence 60/4109.

2.2. The effects of unpredictable food on baseline corticosterone

To check the effects of the food treatment on baseline corticosterone (the main glucocorticoid in birds), we sampled subsets of randomly selected birds from both replicates two weeks after the first treatment exposure period (which occurred between the start of the treatment at 152 ± 15 days of age until the first interruption of the treatment at 188 ± 13 days of age — mean ± SD for all; replicate 1: n = 12 control and 14 unpredictable food birds; replicate 2: n = 14 control and 15 unpredictable food birds). Then, during the second treatment exposure period (which occurred between 283 ± 14 days of age until the second interruption of the treatment at 408 ± 12 days of age — mean ± SD for all), we sampled a random subset of birds from replicate 2 (n = 29 control and 30 unpredictable food birds — for logistic reasons we could not sample replicate 1 birds) after two weeks of treatment; the majority of these birds (n = 25 control and n = 28 unpredictable food birds) were blood sampled again at six weeks into the treatment period (n = 27 control and 28 unpredictable food birds). At the end of a period of food withdrawal in the experimental birds, birds in both treatment groups were blood sampled (~75 μl) within 3 min of entering the room to obtain a baseline blood sample (Wingfield et al., 1982). We recorded bleed time from each individual bird. Blood samples were