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Food access modifies GnIH, but not CRH, cell number in the hypothalamus in a female songbird

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ABSTRACT

Food deprivation or restriction causes animals to mount a stereotypical behavioral and physiological response that involves overall increases in activity, elevated glucocorticoid production, and (often) inhibition of the reproductive system. Although there is increasing evidence that these responses can differ in their degree or covariation between the sexes, most studies to-date on food restriction/deprivation have focused on male songbirds. We therefore aimed to characterize the behavioral, physiological, and neuroendocrine response to acute food deprivation in a female songbird using a nomadic species, the zebra finch. We quantified behavior during a 6.5 h food deprivation and then measured physiological and neuroendocrine responses of female birds at the 6.5 h timepoint. Within 1 h of acute food deprivation, female zebra finches increased foraging behaviors, and after 6.5 h of food deprivation, females lost 5% of their body mass, on average. Change in body mass was positively associated with elevated corticosterone and (contrary to findings in male zebra finches) negatively related to the number of gonadotropin inhibitory hormone-immunoreactive cells in the hypothalamus. However, there was no effect of food deprivation on corticotropin releasing hormone-immunoreactive cells in the hypothalamus. There was also no relationship between corticotropin releasing hormone-immunoreactive cell number and circulating corticosterone. Our results are consistent with the hypothesis that neuroendocrine responses to food deprivation differ between male and female songbirds. Future studies should work to incorporate sex comparisons to evaluate sex-specific neuroendocrine responses to acute stress.

1. Introduction

Organismal fitness is a function of both reproduction and survival, and thus the trade-off between investment of energy into reproduction versus self-maintenance remains fundamental to how we understand life history variation in and among species (Stearns, 1989). While large-scale variation in this trade-off determines pace of life syndromes and life history strategies (Ricklefs and Wikelski, 2002), the trade-off also operates on short timescales as animals organize their behavior and physiology around temporal variation in resource availability within the environment.

Reproductive-self-maintenance trade-offs are mediated in part by neuroendocrine circuits that orient whole-organism performance towards one investment strategy over the other. The hypothalamo-pituitary-adrenal (HPA) and hypothalamo-pituitary-gonadal (HPG) neuroendocrine axes are two core integrator and effector systems that coordinate each investment strategy at the organismal level. Whereas acute, intense activation of the HPA axis promotes physiology and behaviors that support self-maintenance, in part through the production of glucocorticoids, the HPG axis supports reproductive functions through the production of reproductive hormones (testosterone, estradiol, etc.). In addition to responding directly to cues about resource access, these two axes interact with one another: activity at each level of the HPA axis has been shown to inhibit various components of HPG axis (see reviews including Acevedo-Rodriguez et al., 2018; Deviche et al., 2017; Rudolph et al., 2016).

The sensitivity of the HPA and HPG axes to environmental stressors differs between the sexes. For example, acute restraint stress modifies

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expression of gonadotropins in the pituitary of female but not male zebra finches (Ernst et al., 2016). Further underscoring this observation, of over 2000 genes that were differentially expressed in response to acute restraint stress along the dove HPG axis, only 49, or fewer than 3%, were similarly regulated in males and females (Calisi et al., 2018). The HPG axis also responds in a sex-dependent manner to chronic and early-life stressors (Dickens and Bentley, 2014; Schmidt et al., 2014). These sex-dependent responses likely reflect fundamental differences in how each sex invests in reproduction, and they will shape how flexible each sex is in the face of environmental stressors.

There is a wealth of data available describing the neuroendocrine, behavioral, and physiological response to food deprivation or restriction in songbirds (Budki et al., 2009; Cornelius et al., 2018, 2010; Davies et al., 2015a,b; Fokidis et al., 2012; Khalilieh et al., 2012; Krause et al., 2017; Lendvai et al., 2014; Lynn et al., 2015, 2010, 2003; Ritschard and Brumm, 2012), but most experiments to date have used only males or excluded sex-specific analyses. To help fill this gap, we chose to focus on the behavioral and neuroendocrine response of female zebra finches to food deprivation. We hypothesized that food deprivation modifies behavior and physiology through concomitant changes to physiological markers of energy status and to the balance of production and release of HPG- and HPA-axis-associated neuroendocrine hormones in the hypothalamus. We were particularly interested in the rapid and sustained increase in foraging behavior that occurs following food deprivation. We first predicted that food deprivation would modify the concentration of energy substrates in the blood, circulating corticosterone, and body mass. We also predicted that food deprivation would alter the number of hypothalamic neurons immunoreactive for two important upstream regulators of the HPG and HPA axes: gonadotropin inhibitory hormone (GnIH) and corticotropin releasing hormone (CRH), respectively. GnIH neurons are responsive to food deprivation and other stressors in songbirds (e.g., Dickens and Bentley, 2014; Ernst et al., 2016; Lynn et al., 2015). CRH neurons are seldom examined in the songbird hypothalamus in the context of acute stressors (but see Madison et al., 2018), but CRH is important for activation of the HPA axis (Carsia et al., 1986; Salem et al., 1970). Finally, we predicted that changes in hypothalamic neuropeptides GnIH and CRH would be associated with other physiological signals of food deprivation (concentration of energy substrates, corticosterone, and body mass), reflecting cue integration at the level of hypothalamus.

2. Materials and methods

2.1. Animals

Adult (greater than 180 days of age), female zebra finches were randomly selected from mixed-sex, free-flight aviaries at the UC Berkeley Field Station for the Study of Behavior, Ecology, and Reproduction at the University of California, Berkeley. Females were housed in two aviaries (7 females per aviary), separated from males, and allowed to acclimate for 72 h. Animals were provided with *ad libitum* water and German millet mixed with canary seed as well as normal enrichment (natural eucalyptus branches, lettuce twice per week). Animals were exposed to natural changes in daylength (sunrise, 06:32; sunset, 18:08) and provided with 12:12 supplemental artificial light (07:00–19:00).

2.2. Experimental paradigm

The experiment was carried out between 08:00 and 16:00 on March 7th, 2018. The control group was left undisturbed with *ad libitum* food for the experimental period. Food was removed from the food-deprivation aviary at 09:30 and deprivation lasted for six and a half hours. Behavior data were collected using video cameras in the aviaries. At 16:00, birds were humanely euthanized using deep anesthesia with isoflurane followed by decapitation. Trunk blood samples were collected immediately following decapitation. All birds were collected within 4 min of entering the aviary. Animals were weighed 24 h before the manipulation, and then immediately before termination of the experiment. All animal care and procedures were approved by the University of California Office of Laboratory Animal Care and conducted in accordance with local animal welfare laws and policies.

2.3. Behavioral measures

Cameras were set up within each aviary at the beginning of the experiment (08:00). Within the aviaries, all food and water was located on the floor of the aviary, and eucalyptus branches were suspended ~4 ft above the ground. Birds thus had to move between the canopy and floor to forage. Video cameras were unable capture the full aviary in one frame, so cameras were set up to capture the floor of the aviary. When birds were not visible on the floor (either in food dishes or foraging on the floor itself), they were assumed to be in the canopy. Using video footage, the location of every bird in each aviary (*ad libitum* fed or food deprived) was counted every 5 min for the duration of the experiment. Birds were classified as either being in the canopy, on the floor, or in the food dish. Individuals within an aviary could not be differentiated through this method, and the fact that animals were housed within a single aviary presents non-independence problems for analyzing individual behaviors. We instead focused on behavior of the group for analyses (See 2.9 Statistical Analyses).

2.4. Immunohistochemistry

Brains were fixed in ice-cold 4% paraformaldehyde solution (PFA) for 7 days, cryoprotected using 30% sucrose in 0.1 M phosphate-buffered saline (PBS) for 48 h at 4 °C, and then frozen on dry ice and stored at −80 °C. We collected sections of brain cut at 40 μm using a cryostat (cm3050s, Leica Microsystems, Buffalo Grove, IL, U.S.A.) starting at the appearance of the tractus septomesencephalicus (TrSM) and stopping when the cerebellum presented. Brain slices were stored at −20 °C in ethylene glycol and sucrose-based antifreeze in 24-well plates until all samples were ready for immunohistochemistry (IHC).

We used free-floating immunohistochemistry (IHC) to double label gonadotropin inhibiting hormone (GnIH) and corticotropin releasing hormone (CRH) (see Fig. S1A for a representative image). The protocol for detecting GnIH-ir neurons in zebra finches using the PAC123/124 antibody (antigen sequence: SKIPFSNLPLRF, made in rabbit, Bentley, Berkeley, CA, USA) has been previously validated by our laboratory and others (Perfito et al., 2011; Tobari et al., 2010). To our knowledge, this is the first detection of CRH-ir neurons in zebra finches using PBL rC70 (made in rabbit, Wiley, Salk Institute, La Jolla, CA, USA). Within the hypothalamus, we found CRH-ir staining largely restricted to the paraventricular nucleus, as expected in songbirds (Ball et al., 1989). We found no immunoreactive staining when omitting the primary antibody (see Fig. S1B,C).

All washes were carried out three times (5 min.each) on an orbital shaker (S2020, Orbit P4 Digital Shaker, Labnet International Inc., Edison, NJ, U.S.A.) Brain sections were incubated in 0.3% hydrogen peroxide in methanol (10 min,no shaking), washed in 0.1 M phosphate-buffered saline (PBS, pH 7.4), and then blocked for 1 h at room temperature (blocking solution: 2% normal goat serum [Vector Laboratories, Burlingame, CA, U.S.A.] in 0.2% phosphate-buffered saline with Triton x-100 [PBS-T; BP151, Fisher Scientific, Fairlawn, NJ, U.S.A.]). Brain slices were then incubated with CRH primary antibody (1:6000 in 0.2% PBS-T). After 72 h at 4 °C, brains were washed with PBS-T and incubated with secondary antibody (biotinylated goat anti-rabbit; 1:250 in 0.2% PBS-T, 1 h at room temperature, Vector Laboratories, Burlingame, CA, U.S.A.). After washing with PBS-T to remove the secondary antibody, avidin/biotinylated enzyme complex solution was applied (1 h at room temperature; Vectastain Elite ABC Kit, PK-6100; Vector Laboratories, Burlingame, CA, U.S.A.); slices were

washed again with PBS-T before color development with diaminobenzidine (DAB) peroxidase (SK-4100, Vector Laboratories, Burlingame, CA, U.S.A.). Brains were washed five times in PBS before incubating in GnIH primary antibody (1:5000 in 0.2% PBS-T, 48 h at 4 °C, PAC 123/124). After washing away primary GnIH antibody in 0.2% PBS-T, brains were incubated with secondary antibody (biotinylated goat anti-rabbit; 1:250 in 0.2% PBS-T; 1 h at room temperature), washed again in 0.2% PBS-T, and then amplified in a 1 h incubation with ABC. GnIH-ir cells were visualized using Vector VIP Peroxidase substrate kit (SK-4600, Vector Laboratories, Burlingame, CA, U.S.A.) as per manufacturer's instructions (Vector Laboratories, Burlingame, CA, U.S.A.) after washes with 0.2% PBS-T. Slices were washed in 0.1 M PBS and 0.05 M PBS before mounting onto gelatin-coated slides from 0.01 M PBS. Sides were dried at room temperature for 24 h, cleared with Histoclear (HS2001GLL, Fisher Scientific, Fairlawn, NJ, U.S.A.), and coverslipped with Permount (SP 15–100, Fisher Scientific Inc., Waltham, MA. U.S.A.).

Immunoreactive cells within the paraventricular nucleus of the hypothalamus were manually counted by a researcher who was blind to the experimental treatment groups. Due to technical errors in the sectioning and staining process, hypothalamic cell counts from two individuals could not be collected (both from the *ad libitum* treatment group).

2.5. Blood sample processing

Samples were centrifuged for 10 min at 2500*g* and stored at −80 °C until analysis.

2.6. Glucose ELISA

Glucose Colorimetric Assay was used to measure serum levels according to the protocol outlined by the manufacturer (Cayman Chemical, Ann Arbor, MI, USA). Samples were run in triplicate with an average intrassay CV of 1.04%.

2.7. Uric acid ELISA

Uric Acid Assay was used to measure serum levels according to the protocol outlined by the manufacturer (Eton Biosciences, San Diego, CA, USA). Samples were run in duplicate with an average intraassay CV of 4.82% for all samples.

2.8. Corticosterone ELISA

Circulating corticosterone was quantified using Arbor Assays ELISA kit (Catalog No. K014). Plasma was extracted prior to assay using a modified version of the diethyl ether extraction described by Taff et al. (2019). Briefly, 5 μL of plasma was mixed into 45 μL of distilled water, and then mixed with 250 μL of diethyl ether for 2 min on an orbital shaker at 1200 rpm. Layers were allowed to separate for 5 min and then snap frozen in a −80 °C freezer. The top layer (diethyl ether containing extracted steroids) was poured off and the procedure was repeated. Samples were dried in a SpeedVac and stored at −80 °C until assay. Samples were reconstituted in 130 μL of assay buffer immediately prior to assay. Extraction efficiency was estimated using spike-recovery on a subset of samples: a replicate aliquot of serum from 8 individuals was combined with 500 pg of corticosterone in 5 μL of MilliQ water using diluted standard from the assay kit. Spiked and unspiked sample were extracted at the same time and recovery was calculated as the percent of corticosterone remaining when subtracting the unspiked value from the spiked value (e.g., perfect extraction efficiency would be equivalent to 500 pg). Average recovery across 8 independent estimates was 84 ± 2.3% (Mean ± SE). All samples were assayed in duplicate and average intraassay CV was 5.14%. All samples were run on a single plate. Reported values do not account for extraction efficiency.

2.9. Statistical analysis

Treatment group differences in observations of birds in a given location were analyzed using chi-squared analyses. We first tested whether treatment group influenced the number of observations of birds out of the canopy (in view) during the experiment. Second, we tested whether treatment group influenced observation on the floor versus at food dishes. Finally, we examined whether treatment influenced group size viewed at each observation; observations were binned as either have 1, 2, or 3 or more birds in view.

Mann-Whitney U tests were used to compare the medians between the two groups for uric acid concentration, glucose concentration, mass loss, corticosterone concentration, GnIH-ir and CRH-ir cell body counts. Correlations between outcome variables were analyzed using Spearman's rank correlation rho.

Results were considered significant at $P < 0.05$.

3. Results

Animals in the food deprived treatment were observed out of the canopy more frequently than birds with *ad libitum* food access during the experimental period (Figs. 1, 2A; $X^2 = 6.66$, df = 1, P = 0.009). When in view, birds under food deprivation were more often observed on the floor of the aviary (Fig. 2A, $X^2 = 15.85$, df = 1, $P < 0.001$). Food restricted birds also tended to be observed in larger groups while on the floor compared to birds with *ad libitum* food access (Fig. 2B, Fig. 1C; $X^2 = 23.87$, df = 2, $P < 0.001$), but in similar sized groups at food dishes (Fig. 2B; $X^2 = 3.14$, df = 2, P = 0.21).

There was no difference in median bird mass between treatments prior to the manipulation (W = 37, P = 0.13). Food deprivation caused a median mass loss of 5%, compared to a median of 0.1% mass loss in birds with *ad libitum* food access (Fig. 3A; W = 49, $P < 0.001$). Concordant with mass, median serum corticosterone was more than 13-times higher in food deprived birds compared to *ad libitum* fed birds

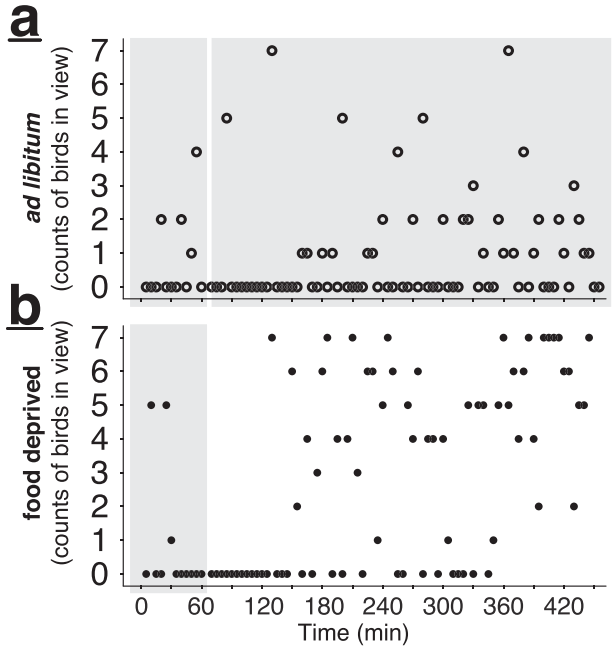

Fig. 1. Acute food deprivation increases activity specifically associated with foraging on the floor. Video footage covered the floor of the aviary, and thus birds can be classed as in view (at food dishes or on the floor) or out of view (in the canopy of the aviary). Shaded area in each panel indicates periods with food available to birds *ad libitum* in each aviary. Panels show counts of birds (maximum of 7 in each aviary) either on the floor or in food dishes at 5 min intervals across the experiment in the *ad libitum* (A) and food deprived (B) aviaries.

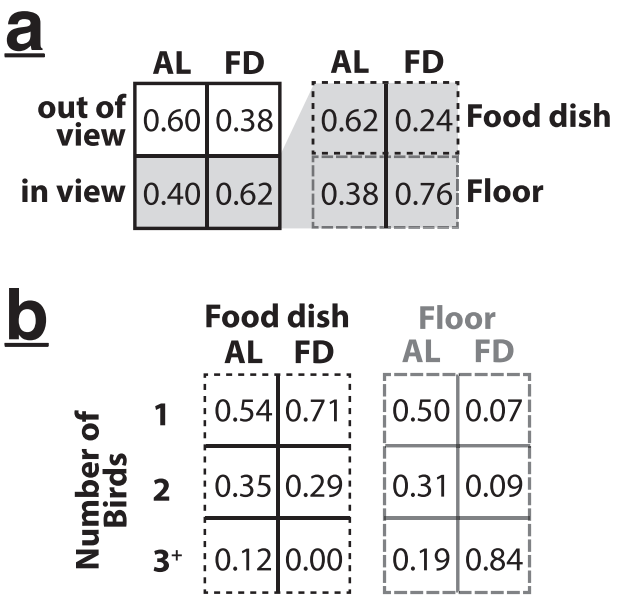

Fig. 2. Contingency tables showing the proportion of observations within each treatment group. (A) The proportion of observations with birds in view (on the floor or at food dishes) was greater in food deprived (FD) aviary compared to the *ad libitum* (AL) group (left table). Furthermore, of the observations with birds in view, animals in the FD treatment were more often observed on the floor as opposed to the at the food dish (right) (B) Although we did not detect a difference between the proportion of observations with group sizes of 1, 2 or 3^+ birds at food dishes between treatments (left), birds were observed on the floor were more often in groups of 3^+ individuals in the FD treatment (right).

(Fig. 3B; W = 4, P < 0.01), and serum corticosterone was negatively correlated with mass loss (Fig. 5A; S = 792, P < 0.01). Neither serum glucose nor uric acid differed between treatment groups (Fig. 3C,D; P = 0.45), and there were no correlations between serum corticosterone and glucose or uric acid (Fig. S2C,D; glucose: S = 476, P = 0.88; uric acid: S = 524, P = 0.61).

There was no difference in the number of CRH-immunoreactive (CRH-ir) neurons within the paraventricular nucleus of the hypothalamus between treatment groups (Fig. 4A; W = 12, P = 0.43), but food deprived birds had fewer hypothalamic GnIH-immunoreactive (GnIH-ir) neurons than *ad libitum* fed birds (Fig. 4B; W = 30, P < 0.05). The number of GnIH-ir neurons was negatively correlated with change in mass (Fig. 5B; S = 70, P < 0.001): birds that lost more mass across the experiment had fewer GnIH-ir neurons. The number of GnIH-ir neurons was also significantly, negatively correlated with corticosterone (Fig. 5C; S = 462, P < 0.04). The number of CRH-ir cells was not correlated with circulating corticosterone (Fig. 5D; S = 236, P = 0.59), the number of GnIH-ir cells (Fig. S2A; S = 250, P = 0.70), or mass loss (Fig. S2B; S = 322, P = 0.70).

4. Discussion

We were interested in exploring the relationships between neuroendocrine circuits and physiological signals that respond to food deprivation in a female songbird. We hypothesized that food deprivation concomitantly alters physiological signals associated with energy status and neuroendocrine hormones in the hypothalamus. Our data suggest that, in female zebra finches, physiological signals of food deprivation are associated with a shift in the balance of production and release of the HPG-axis-associated neuropeptide GnIH. These data provide further evidence that there are there are sex-specific differences in the physiological and neuroendocrine circuits that control an individual's response to acute stressors like food deprivation.

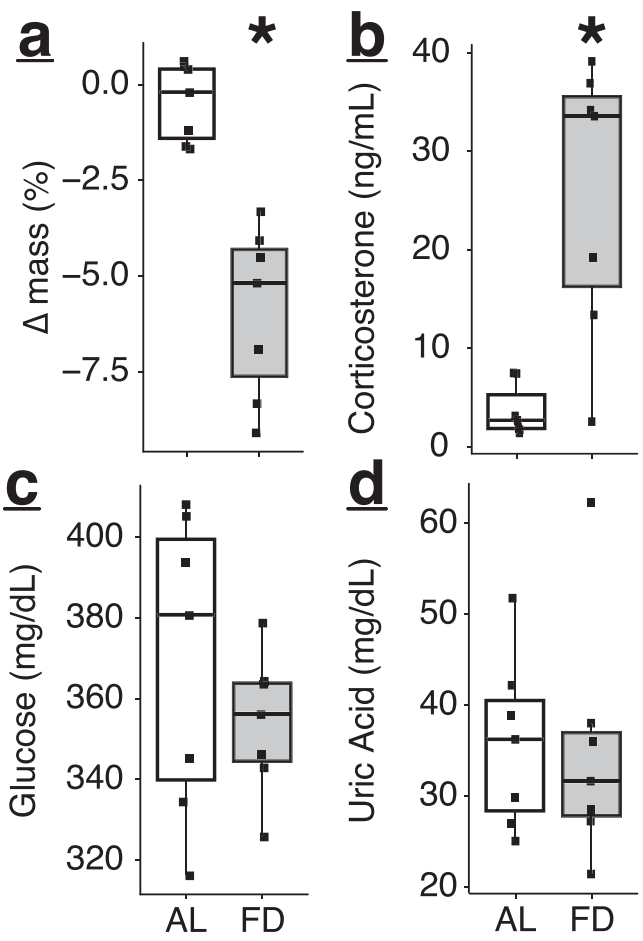

Fig. 3. Food deprived (FD, shaded boxes) birds lost a greater percent of their body mass (A) and elevated circulating corticosterone (B) relative to *ad libitum* (AL, open boxes) fed birds. However, circulating glucose (C) and uric acid (D) do not differ between groups. Each black point represents a single individual. Whiskers in each box-and-whisker plot extend to 1.5 of the interquartile range times the 25th and 75th percentile (the lower and upper quartiles, respectively). The horizontal line in the middle of the box shows the 50th percentile. *P < 0.05, Mann-Whitney U Test.

4.1. The hypothalamic response to food deprivation

We found no effect of food restriction on CRH-ir cell number or any relationship between CRH-ir cell number and other variables (including circulating corticosterone). The absence of change in immunoreactive cell number may reflect that, as in mammals (Mershon et al., 1992), CRH release and production is pulsatile, and thus immunohistochemistry approaches may not be a reliable approach for quantifying changes in CRH neuron activity (Bloom et al., 1982; Cusulin et al., 2013). Thus, even though we were able to detect a large number of CRH-ir cells, rapid production and release may prevent detection of changes via immunohistochemistry. Arginine vasotocin (AVT), another important hypothalamic regulator of HPA axis activity with fewer methodological challenges, may provide additional insight into sex-specificity of acute responses to stress (Kuenzel and Jurkevich, 2010; Nagarajan et al., 2014). Given the fundamental regulatory role of CRH and AVT in HPA axis function, quantifying their activation under acute stress is important for understanding the mechanistic basis of sex-specific stress responses.

Food deprivation was associated with a decrease in hypothalamic GnIH-ir cell number in female zebra finches. In contrast, food deprivation does not affect protein product or transcript abundance of reproductive genes in the hypothalamus of male zebra finches after

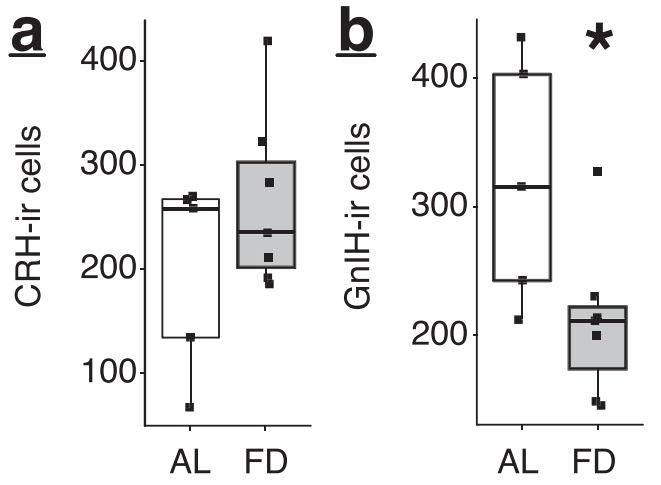

Fig. 4. (A) Food deprived birds (FD, shaded boxes) have similar numbers of corticotropin-releasing hormone-immunoreactive (CRH-ir) cells in the hypothalamus relative to *ad libitum* fed (AL, open boxes) birds. (B) The number of gonadotropin inhibitory hormone-immunoreactive cells in the hypothalamus is lower in food deprived birds compared to those with *ad libitum* food access. Each black point represents a single individual. Whiskers in each box-and-whisker plot extend to 1.5 of the interquartile range times the 25th and 75th percentile (the lower and upper quartiles, respectively). The horizontal line in the middle of the box shows the 50th percentile. *P < 0.05, Mann-Whitney U Test.

similarly lengthy food deprivations (Ernst, 2015; Lynn et al., 2015). The difference in hypothalamic responses between male and female zebra finches may reflect sex-specific strategies for regulating the reproductive axis in response to stress. Male zebra finches do show differential regulation of genes in the testis during food deprivation (Lynn et al., 2015), leading Lynn and coauthors to suggest that suppressing reproduction at the level of the testes may allow males to rapidly activate the reproductive system when conditions improve. Following this idea, hypothalamic responses shown in females as part of this study may be indicative of more sustained or complete reproductive inhibition via regulation of pituitary function (e.g., gonadotropin production and release, as suggested by Ernst et al., 2016). In the same line of thinking, Calisi et al. (2018) found more extensive differential regulation at all levels of the HPG axis in females compared to males in response to acute restraint stress, which may suggest that the female HPG axis is overall more responsive to stressors.

Hypothalamic GnIH has also been implicated in regulating feeding behavior in birds (Tachibana et al., 2008, 2005; Tsutsui and Ubuka, 2016). The food deprivation-dependent reduction in GnIH-ir cells in the hypothalamus coupled with the correlation between GnIH-ir cell number and mass change across groups, shown here, suggest that loss of body condition may stimulate food seeking through GnIH signaling in the hypothalamus. The mechanism by which body condition is communicated to GnIH neurons remains unclear, but it could be important for understanding sex-based differences in the response of GnIH to stressors.

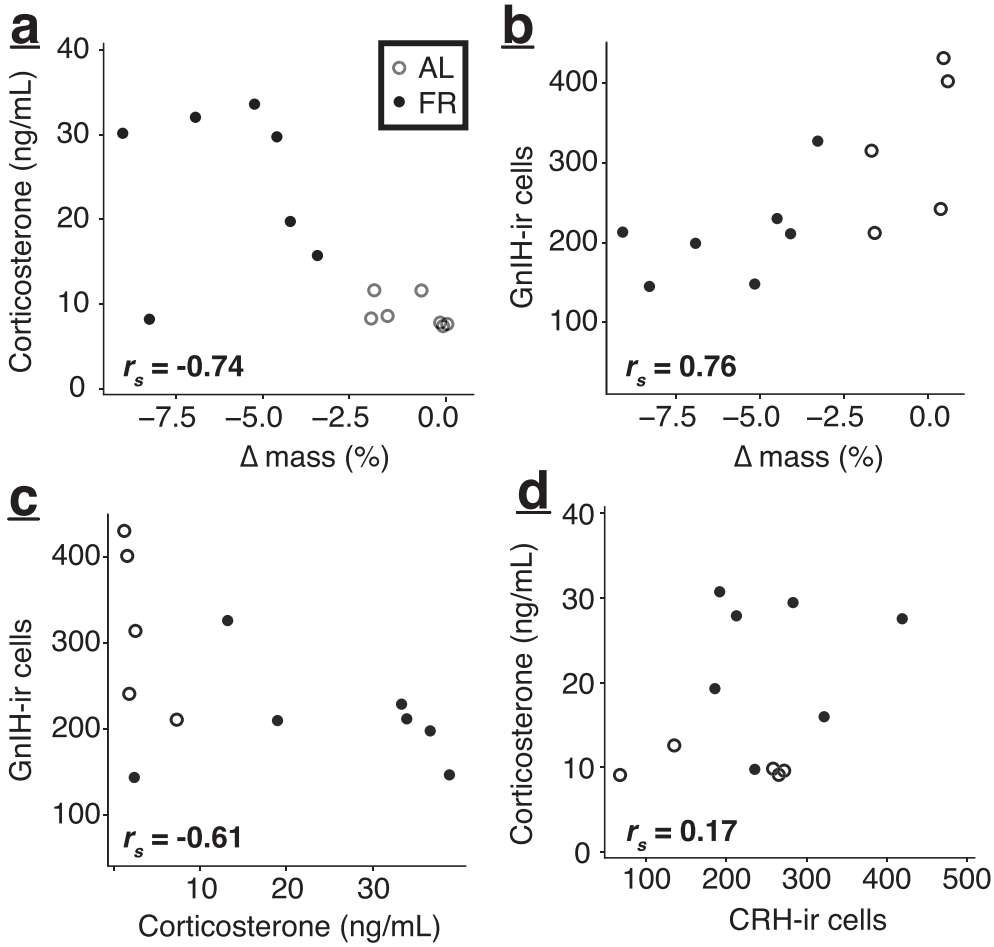

Fig. 5. Correlations among phenotypic measures associated with the response to food deprivation in female zebra finches. Birds in the *ad libitum* (AL) group are indicated by open circles, and birds in the food deprivation (FD) group are indicated by filled circles. Spearman's rank correlation rho is indicated in the bottom left of each panel (r_s). Percent mass loss is correlated with (A) serum corticosterone and (B) gonadotropin inhibitory hormone-immunoreactive (GnIH-ir) cell number. Serum corticosterone was correlated with (C) GnIH-ir cell number but not with (D) CRH-ir cell number.

4.2. Circulating factors responding to food deprivation

Fasting animals progress through a series of metabolic states or stages that can be differentiated by relative concentrations of plasma metabolites used to fuel energy use (Alonso-Alvarez and Ferrer, 2001; Castellini and Rea, 1992; Le Maho et al., 1981, see also McCue, 2010). In birds, circulating glucose is maintained until individuals near complete starvation, and thus any decrease in glucose would suggest that birds were nearing death. In contrast, uric acid increases during earlier stages of starvation as fat is depleted and animals begin to use protein as a primary fuel source (Lindgård et al., 1992). Baseline corticosterone also rises as lipids are depleted (e.g., Krause et al., 2017). Given that neither glucose nor uric acid concentration were altered by food deprivation, but corticosterone was significantly elevated, birds in our study were likely in early physiological stages of starvation where they are primarily on relying on lipids for fuel.

Glucose and uric acid concentrations in the blood can also vary among life history stages and decrease in response to acute stress (Deviche et al., 2016, 2014), however our study suggests that mechanisms induced by extended food deprivation are sufficient to restore both energy substrates to homeostatic levels in female zebra finches. The concentration of these substrates over an extended period therefore is unlikely to drive behavioral or phenotypic changes sustained under food deprivation. Instead, turnover or other by-products of lipid and protein catabolism are probably more useful measures for understanding how neuroendocrine axes use metabolic information to regulate behavioral and physiological responses.

Sustained, elevated corticosterone, as we report in this experiment, is a core component of the response to food restriction in zebra finches. The correlation between circulating corticosterone and mass loss further reinforces that corticosterone is connected to body condition (as opposed to simply the perception of low food availability) over relatively acute timescales. We observed one individual that did not have elevated corticosterone despite substantial mass loss. The outlier response is particularly striking within the correlation plots including corticosterone (Fig. 5A). Previous work in male birds from the same colony showed a bimodal response to food restriction in which 3 of 10 birds did not have elevated corticosterone after a 10 h fast (Lynn et al., 2015). In our study, the individual with low corticosterone did not have outlier values in circulating energy substrates or immunoreactive cell number in the hypothalamus. We therefore cannot determine whether the bimodal corticosterone concentrations in food-deprived individuals suggests that some animals are reaching homeostatic limit at which point they lose the ability to maintain homeostasis or whether the bimodal response reflects fundamentally different responses to food deprivation among animals. The repeated observation of this bimodal response suggests that further attention to characterizing these differences is warranted.

4.3. Behavioral response to food deprivation

When food becomes inaccessible, animals rapidly shift their behavior and increase activity (Krause et al., 2017; Lynn et al., 2003). We show that the increased activity is specific to activity associated with foraging on the floor (Fig. 1, 2), as is appropriate when a fixed food source is not reliable or available. Although it was not a goal of the experiment as designed, we found that food restricted birds foraged on the floor in larger groups than *ad libitum* fed birds (see Results). Conversely, (Crino et al., 2017) found that females in poorer body condition tended to feed with fewer companions relative to females in better condition. Sociality associated with foraging behavior may therefore differ from sociality that occurs as part of feeding at a fixed food source; these differences suggest that feeding and foraging are not interchangeable terms, especially for organisms that utilize patchy resources, like zebra finches.

5. Conclusions

Contrary to previous work in male zebra finches, we found that the number of hypothalamic gonadotropin inhibitory hormone-immunoreactive (GnIH-ir) neurons decreased in response to food deprivation concordant with mass loss. Our results further support sex-specific responses to stressors, suggesting that sex comparisons are important to understanding the complex neuroendocrine and physiological circuits that coordinate acute stress responses.

CRediT authorship contribution statement

Kathryn Wilsterman: Conceptualization, Formal analysis, Investigation, Project administration, Writing - original draft, Writing - review & editing. **Mattina M. Alonge:** Investigation, Validation, Writing - review & editing. **Xinmiao Bao:** Investigation, Validation, Writing - original draft, Writing - review & editing. **Kristin A. Conner:** Investigation, Validation, Writing - original draft, Writing - review & editing. **George E. Bentley:** Conceptualization, Investigation, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2020.113438.

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