Physiology & Behavior 133 (2014) 141–151

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Female-biased anorexia and anxiety in the Syrian hamster

John L. Shannonhouse [a], Li An Fong [b], Bryan L. Clossen [a], Ross E. Hairgrove [b], Daniel C. York [c], Benjamin B. Walker [b], Gregory W. Hercules [b], Lauren M. Mertesdorf [d], Margi Patel [b], Caurnel Morgan [a,b,c,*]

[a] Texas A&M Institute for Neuroscience, Texas A&M University, College Station, TX 77843, United States
[b] Department of Nutrition & Food Science, Texas A&M University, College Station, TX 77843, United States
[c] Department of Psychiatry, Weill Cornell Medical College, New York, NY 10021, United States
[d] Department of Biochemistry, Texas A&M University, College Station, TX 77843, United States

HIGHLIGHTS

- Social separation induced anorexia and anxiety with female biases.
- Anorexic and anxious phenotypes dissociable from HPA activation
- HPA activity in socially housed controls dissociable from social stress
- Neural cytokines were implicated in female-biased anorexia and anxiety.

ARTICLE INFO

Article history:
Received 25 April 2014
Received in revised form 7 May 2014
Accepted 16 May 2014
Available online 24 May 2014

Keywords:
Corticosteroids
Corticotropin-releasing factor
Cytokines
Metabolism
Social stress

ABSTRACT

Anorexia and anxiety cause significant mortality and disability with female biases and frequent comorbidity after puberty, but the scarcity of suitable animal models impedes understanding of their biological underpinnings. It is reported here that in adult or weanling Syrian hamsters, relative to social housing (SH), social separation (SS) induced anorexia characterized as hypophagia, weight loss, reduced adiposity, and hypermetabolism. Following anorexia, SS increased reluctance to feed, and thigmotaxis, in anxiogenic environments. Importantly, anorexia and anxiety were induced post-puberty with female biases. SS also reduced hypothalamic corticotrophin-releasing factor mRNA and serum corticosteroid levels assessed by RT-PCR and RIA, respectively. Consistent with the view that sex differences in adrenal suppression contributed to female biases in anorexia and anxiety by disinhibiting neuroimmune activity, SS elevated hypothalamic interleukin-6 and toll-like receptor 4 mRNA levels. Although corticosteroids were highest during SH, they were within the physiological range and associated with juvenile-like growth of white adipose, bone, and skeletal muscle. These results suggest that hamsters exhibit plasticity in bioenergetic and emotional phenotypes across puberty without an increase in stress responsiveness. Thus, social separation of hamsters provides a model of sex differences in anorexia and anxiety during adulthood and their pathogeneses during adolescence.

1. Introduction

Emotional states influence food intake, weight gain, metabolism, food preferences, and/or taste preferences in humans, mice, rats, and hamsters [1–8]. Despite the extent of this phylogenetic conservation, relatively few reports have focused on emotional factors in energy balance [2,9–11]. Moreover, such studies have used male subjects overwhelmingly. Due to the scarcity of female or sex-comparative animal models, little is known about the mechanisms underlying sex differences in bioenergetic and emotional interactions [12]. This knowledge gap is significant because it limits the understanding of conserved mechanisms that are crucial for survival.

Food deprivation has been shown to exert anxiolytic-like effects in rats [13–15]. By contrast, it has been shown in hamsters to induce anxiogenic-like effects [16] and increase responsiveness to anxiolytic drugs [17,18]. Adolescent activity-based anorexia (ABA) uses restricted feeding and hyperactivity to model core features of anorexia nervosa in adulthood. In female rats, ABA also increased anxious behavior in the open field test [19]. In female mice, a low-anxiety strain reduced their activity, relative to a high-anxiety strain, during ABA [20]. Adolescent mice also exhibited an anxiety-related increase in hippocampal γ-aminobutyric acid A receptor signaling [21]. To our knowledge, ABA has not been little used in males.

* Corresponding author at: 218C Kleberg Center, Department of Nutrition & Food Science, Texas A&M University, College Station, TX 77843-2253, United States. Tel.: +1 979 458 1849; fax: +1 979 458 3704.
E-mail address: camorgan@tamu.edu (C. Morgan).

http://dx.doi.org/10.1016/j.physbeh.2014.05.019
0031-9384/

These findings provide bases for models to study bidirectional relationships between eating disorders and emotional disorders with two caveats. First, eating disorders and anxiety disorders have female biases [19]. By contrast, in adult rats the anxiolytic response to food deprivation, and the anxiogenic effects of ABA in mice and rats, occurred in females [13–15], but they have not been shown in males. Additionally, little is known about anxious behaviors in female hamsters. Second, these models require non-volitional food deprivation or restriction to alter anxious behaviors. Importantly, the negative impact on behavior and physiology that is associated with the lack of control over stressors is conserved in humans, rats, mice, and hamsters [22–26]. Therefore, models using volitional food restriction would be useful.

Loss of social contact can induce anxiety and anorexia in humans [27–30], and it might be used to generate animal models of separation-induced anxiety and anorexia with volitional food restriction. Social separation of male rats increased anxious behaviors in the light–dark and elevated plus maze tests, and it induced an anxiogenic-like reduction of cAMP response element-binding protein activity in the nucleus accumbens [31–33]. Social separation of male mice has been shown to induce anxiolytic-like effects in the elevated plus maze, and by increasing limbic 5α-reductase-1 mRNA levels [34–36]. Little has been reported, however, on the effects of social separation in female rats and mice. In prairie voles, assessed in the elevated plus maze, social separation was anxiogenic in females and anxiolytic in males [37,38]. Therefore, further research is warranted to identify models of separation-induced anxiety that are robust in females and mild in males. Further study is also warranted to understand potential sex differences and effects of social separation on food intake [39,40].

The present study was designed to establish a dual model of separation-induced anorexia and anxiety using Syrian hamsters. Three lines of evidence suggested the plausibility of such a model. First, we have shown that social separation of male hamsters decelerated food intake and weight gain [41]. This finding is supported by reports of reduced cumulative food intake and weight gain in hamsters [42–44]. Second, we have shown that food deprivation induced anxiogenic-like effects in hamsters [16], and this finding is supported by reports of food deprivation elevating behavioral sensitivity to anxiolytic drugs in hamsters [17,18]. Third, previous studies of hamsters have indicated sex differences in energy balance and in bioenergetics- and emotionality-regulating corticosteroid responses to aversive stimuli [44–46]. These findings suggest that separation-induced volitional food restriction might induce anxiety in hamsters with sex differences. Accordingly, the hypothesis that social separation induces female-biased anorexia and anxiety was formulated and tested herein. The rationale for this study is that it has potential to provide a model of bioenergetic and emotional interactions that are crucial for survival.

2. Materials and methods

2.1. Subjects

Syrian golden hamsters (*Mesocricetus auratus*) of the Lake View Gorge strain (Charles River, Kingston, NY) were purchased for use at Weill Cornell Medical College, or they were bred in the Kleberg Laboratory Animal Facility at Texas A&M University. A 14 h:10 h light–dark schedule (lights on at 0600 h) at 23 ± 3 °C was kept. LabDiet 5001 (Purina Mills, Richmond, IN) and water were provided *ad libitum*. Each cage was supplied with Sani-Chips (Murphy's Products, Monteville, NJ) and Nestlets nesting material (Ancare, Bellmore, NY).

2.2. Experimental designs

Hamsters were housed 2–4/cage during same-sex social housing (SH) or 1/cage during social separation (SS). *Study 1*. Adult female and male hamsters were assessed for anorexia during SH or SS. Experiment 1.1. Weight gain and food intake ($n = 8$) were assessed between ages 10 and 20 weeks. Experiment 1.2. Metabolic activity ($n = 7$–10) was assessed after 8 weeks at the peak values for anorexia in experiment 1.1. Experiment 1.3. White adipose tissue weights, body length, tibia length, tibia weight, and anterior tibialis weight ($n = 6$) were assessed after 10 weeks. Experiment 1.4. Hypothalamus, adrenal, and plasma samples ($n = 5$–8) were collected from experiment 1.1 and assessed for neuroendocrine and neuroimmune activities. *Study 2*. Adult female and male hamsters were assessed for anxiety during SH or SS. Experiment 2.1. A subset of hamsters from experiment 1.1 were assessed for behavior in the anxiety-related feeding/exploration conflict (AFEC) test ($n = 6$) after 2–10 weeks. Experiment 2.2. Behavior in the open field test ($n = 6$) was assessed after 8 weeks near the peak values for anxiety in experiment 2.1. *Study 3*. Female and male hamsters were assessed for anorexia and anxiety after juvenile SH ($n = 4$–7) or SS ($n = 4$–5) between postnatal day 29 (PD29) and PD56. Experiment 3.1. Weight gain, food intake, and feed efficiency were assessed until PD56. Experiment 3.2. Behavior in the AFEC test was assessed at PD56. Experiment 3.3. Hypothalamic expression was assessed at PD56. Territorial aggression was minimized by using littermates for SH, and screening for over-aggressiveness twice daily from age 6 weeks. With these observation regimens, it was not necessary to separate any cage mates. Procedures used were approved by the Institutional Animal Care and Use Committees.

2.3. Biometrics

Food intake and weight gain were assessed at the time of regular cage changes during the light phase. Food intake was assessed by combining the weight of chow in the hopper and bedding (after voluntary ejection from the cheek pouches as necessary) to the nearest 0.1 g. During SH, food intake was assessed by dividing the amount determined as above by the number of hamsters per cage. Feed efficiency was calculated as weight gain (g) per food intake (kg). Tissues were excised *post mortem*, and weighed to the nearest 0.1 g (adipose) or 1 mg (adrenals, spleen, tibia, and anterior tibialis). Body and tibia lengths were measured *post mortem*.

2.4. Anxiety-related feeding/exploration conflict (AFEC) test

As we have described [16], individual subjects were transferred to 45 × 20 × 20 cm (L × W × H) polycarbonate test cages. The test food, graham cracker (Nabisco, East Hanover, NJ), was presented overhead in a spring-loaded utility clamp. Intervals between the presentation and sniffing the test food (approach latency) and biting it (feed latency) were timed with a stopwatch by a treatment-blind scorer. To control for potential non-emotional (i.e., olfactory, appetitive, consummatory, and motoric) factors, latencies were also measured after return to the home cage. Testing occurred between 1000 h and 1600 h in a behavior room. Food was removed from the home cage 90 min before testing. For 7 days before testing, subjects were habituated daily with ~30 mg of test food.

2.5. Open field test

Each subject was tested in a 60 cm^2 polyvinyl chloride chamber with a marked center area and four peripheral areas that were 12 cm from the 40-cm walls. Behaviors were recorded for 30 min and scored treatment-blind. Anxiety was assessed by thigmotaxis (avoidance of the center), as characterized previously for hamsters [47–49]. Specifically, the time spent in the anxiogenic center area and numbers of center entries (i.e., all paws inside) were scored. The percentage of center time was calculated as center time / (center plus periphery times) × 100. The percentage of center activity was calculated as center entries / (center plus periphery entries) × 100. Locomotor activity was assessed by numbers of peripheral area entries.