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Sex differences in motivational responses to dietary fat in Syrian hamsters

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HIGHLIGHTS

- Social separation induces female-biased motivational disruption and anorexia.
- High-fat feeding reduces motivational disruption in females only.
- Novel assay, reward investigational preference (RIP) test, for anhedonia
- Improved performance in the RIP test requires chronic antidepressant treatment.
- RIP test distinguishes depressive, anxious, appetitive, and consummatory, behaviors.

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ABSTRACT

Women are more likely than men to exhibit motivational disorders (e.g., anhedonia and anxiety) with limited treatment options, and to overconsume high-fat "comfort foods" to improve motivational disruptions. Unfortunately, neurobiological underpinnings for sex differences in motivational disruptions and their responses to dietary fat are poorly understood. To help bridge these fundamental knowledge gaps, we assessed behavioral and neurobiological responses to dietary fat in a hamster model of female-biased motivational lability. Relative to social housing, social separation reduced hedonic drive in a new behavioral assay, the reward investigational preference (RIP) test. Fluoxetine or desipramine treatment for 21, but not 7, days improved RIP test performance. Pharmacologic specificity in this test was shown by non-responsiveness to diazepam, tracazolate, propranolol, or naltrexone. In the anxiety-related feeding/exploration conflict (AFEC) test, social separation worsened latency to eat highly palatable food under anxiogenic conditions, but not in home cages. Social separation also reduced weight gain, food intake, and adiposity while elevating energy expenditure, assessed by caloric efficiency and indirect calorimetry. Furthermore, chronic high-fat feeding improved anhedonic and anxious responses to separation, particularly in females. In the motivation-influencing nucleus accumbens, females, but not males, exhibited a separation-induced anxiety-related decrease in *Creb1* mRNA levels and an anhedonia-related decrease in *ΔFosb* mRNA levels. Consistent with its antidepressant- and anxiolytic-like effects on behavior, high-fat feeding elevated accumbal *Creb1* and *ΔFosb* mRNA levels in females only. Another accumbal reward marker, *Tlr4* mRNA, was elevated in females by high-fat feeding. These results show that social separation of hamsters provides a novel model of sex-dependent comorbid anhedonia, anxiety, and anorexia, and implicate accumbal CREB, ΔFosB, and TLR4. Moreover, the results validate a new assay for chronic antidepressant efficacy.

1. Introduction

Beneficial effects of high-fat foods on motivational disruptions in humans presumably contribute to their designation as "comfort foods", but the extent to which sex plays a role has not been reported extensively. Interestingly, sex differences in the motivation and preference to eat high-fat foods are thought to contribute to the prevalence of obesity in women more than it does in men [1,2]. In studies that controlled for genetic background with monozygotic twins, obesity was associated with restrictive eating, frequent snacking, and attempted restriction of high-fat foods [3,4]. Furthermore, obese women have self-reported more vulnerability to eating "comfort foods" than normal-

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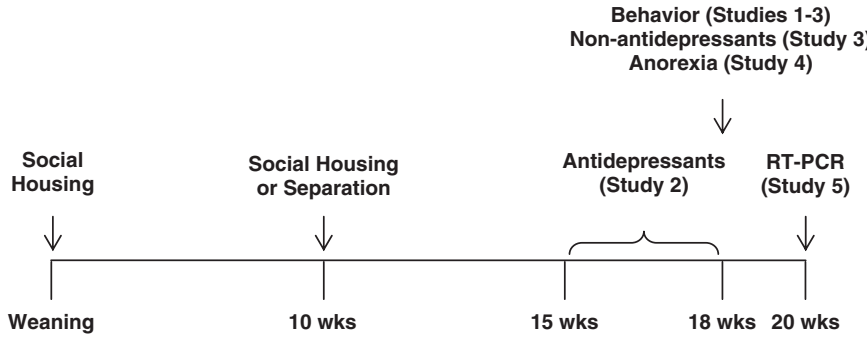

Fig. 1. Timelines for studies. Timelines are shown for the social manipulations and administration of drugs, as well as assessments of motivated behaviors, anorexia, and accumbal expression of target genes.

weight women [4]. Paradoxically, women have been found to exhibit more dietary restraint, emotional eating, and binge eating than men [5]. Investigation in this area is important because female biases are established for eating disorders and overconsumption of high-fat foods [6,7], as well as motivational disorders [8,9], but their biological underpinnings are not. Anhedonia and anxiety are thought to represent neural dysfunctions in positive and negative motivation, respectively [10,11], and they are frequently comorbid with eating disorders [12,13].

Previous rodent studies have shown that dietary fat influences depression- and anxiety-related behaviors. In adult Sprague Dawley rats, subjected to maternal separation as pups, high-fat feeding exerted anxiolytic effects, particularly in males [14,15]. Similar anxiolytic effects of dietary fat have been demonstrated in male Sprague Dawley rats without maternal separation [16]. Additionally, binge eating of high-fat food prevented anxiety after its discontinuation in rats [17]. Interestingly, chronic high-fat feeding fat increased the immobility of male and female Wistar rats in the forced swim test, which is related to psychomotor inhibition, a core symptom of depression in humans [18]. In male C57BL/6 mice, high-fat feeding attenuated stress-induced anxiety and immobility, but not anhedonia [19]. In male CD-1 mice, however, high-fat feeding exerted antidepressant-like effects, but also anxiogenic effects [20]. Discontinuation of high-fat diet worsened anxious and reward behaviors in mice, and it increased their willingness to endure anxiogenic conditions to regain access to high-fat chow [21]. Species, strain, and sex differences notwithstanding, these findings suggest that dietary fat can improve emotionality under certain experimental conditions.

There is mounting evidence that high-fat consumption exerts rewarding effects through actions in the nucleus accumbens, which is an integral component of the mesolimbic reward system and important for bioenergetic and emotional integration. This limbic structure influences depression-related, anxious, and reward states in rats, mice, and humans [22–24], as well as reward behaviors and food preferences in hamsters [25,26]. In rats, a high-fat meal induced greater release of accumbal dopamine, which is a potent reward signal [27]. Studies assessing the correlation to ΔFosB expression or effects of ΔFosB overexpression suggest that this transcription factor increases motivation for high-fat reward, and promotes energy conservation and adiposity [28–30]. Mice overexpressing ΔFosB in the nucleus accumbens also exhibited an enhanced reward response and anxiety following high-fat withdrawal [31]. Furthermore, chronic high-fat feeding in Long Evans rats reduced the accumbal activation of an anxiogenic signal, phosphorylated CREB [32], and in mice ΔFosB-overexpression prevented a decrease in accumbal CREB phosphorylation [31]. In the latter two studies, however, reward behavior was not assessed.

Hypothalamic expression of the cytokine, TLR4, has been implicated as a mediator in the stimulatory effects of high-fat diet on neuronal inflammation, apoptosis, and insulin resistance [33–35]. We have shown that the elevation of hypothalamic *Tlr4* mRNA levels is associated with separation-induced anxiety and anorexia in hamsters [36]. Additionally, TLR4 activation has been shown to mediate drug reward [37–40], but the neuroanatomic site of action has not been established. Because CREB and ΔFosB play opposing roles in the accumbens versus other brain regions [41–44], it is plausible that accumbal *Tlr4* expression increases with reward. Therefore, we explored that possibility in the present study.

The studies cited above collectively illustrate the need for a clinically relevant model to test the hypothesis that modulation of dietary fat contributes to sex differences in motivational states. We have shown previously that, relative to social housing, social separation induced anorexia and anxiety with female biases in Syrian hamsters [36,45,46]. In the present study, we sought to determine whether social separation also induces anhedonia with female bias using a new assay, the reward investigational preference (RIP) test. Importantly, we also sought to determine whether dietary fat reduces anhedonic and anxious behaviors with sex differences.

2. Methods and materials

2.1. Subjects

Syrian golden hamsters (*Mesocricetus auratus*) of the Crl:LVG(SYR) strain (Charles River, Kingston, NY) were purchased for use at Weill Cornell Medical College, or they were bred for up to three generations in the Kleberg Laboratory Animal Research Facility at Texas A&M University. A 14 h:10 h light–dark schedule (lights on at 0600 h) and temperature of 23 ± 3 °C were kept. Low-fat diet, LabDiet 5001 (Purina Mills, Richmond, IN), high-fat diet, D12451 (Research Diets, New Brunswick, NJ), and tap water were provided ad libitum. Procedures used in this study were approved by the Institutional Animal Care and Use Committee.

2.2. Experimental designs

Hamsters were housed 2–4/cage during same-sex social housing (SH) after weaning. Some were housed 1/cage during social separation (SS) at age 10 weeks (see Fig. 1 for timelines of the studies and time courses for drug treatments). Territorial aggression was minimized by

Table 1
PCR primers and conditions.

Gene	Primer sequence	Conditions
Actb	Fwd: GGTATGGAATCCTGTGGCATCCATGA Rev.: ACTCCTGCTTGCTGATCCACATCT	24 cycles, T_M = 93 °C
Creb1	Fwd: ACAGATTGCCACATTAGNCCAGGTA Rev.: TCCACAGACTCCTGTGAATCTTCACT	33 cycles, T_M = 92 °C
ΔFosb	Fwd: GGAGGGTTCGCAGAGAGAGAAACAA Rev.: CCGAGGACTTGAACTTCACTCGG	35 cycles, T_M = 94 °C
Tlr4	Fwd: CTCCCTGAGACCTGAAAGCTTGGAT Rev.: GGTGTAGACCCTGATATGCCTTGTCTT	35 cycles, T_M = 91 °C