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Palatable Food Affects HPA Axis Responsivity and Forebrain Neurocircuitry in an Estrous Cycle-specific Manner in Female Rats

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Abstract—Eating palatable foods can provide stress relief, but the mechanisms by which this occurs are unclear. We previously characterized a limited sucrose intake (LSI) paradigm in which twice-daily access to a small amount of 30% sucrose (vs. water as a control) reduces hypothalamic–pituitary–adrenocortical (HPA) axis responses to stress and alters neuronal activation in stress-regulatory brain regions in male rats. However, women may be more prone to 'comfort feeding' behaviors than men, and stress-related eating may vary across the menstrual cycle. This suggests that LSI effects may be sex- and estrous cycle-dependent. The present study therefore investigated the effects of LSI on HPA axis stress responsivity, as well as markers of neuronal activation/plasticity in stress- and reward-related neurocircuitry in female rats across the estrous cycle. We found that LSI reduced post-restraint stress plasma ACTH in female rats specifically during proestrus/estrus (P/E). LSI also increased basal (non-stress) FosB/deltaFosB- and pCREB-immunolabeling in the basolateral amygdala (BLA) and central amygdala specifically during P/E. Finally, Bayesian network modeling of the FosB/deltaFosB and pCREB expression data identified a neurocircuit that includes the BLA, nucleus accumbens, prefrontal cortex, and bed nucleus of the stria terminalis as likely being modified by LSI during P/E. When considered in the context of our prior results, the present findings suggest that palatable food reduces stress responses in female rats similar to males, but in an estrous cycle-dependent manner. Further, the BLA may contribute to the LSI effects in both sexes, whereas the involvement of other brain regions appears to be sex-dependent. © 2018 Published by Elsevier Ltd on behalf of IBRO.

Key words: sex differences, sucrose, ACTH, corticosterone, basolateral amygdala, nucleus accumbens.

INTRODUCTION

Obesity is one of the largest public health issues in modern times. Over 68% of adults in the United States are overweight or obese, and this number continues to grow (Flegal et al., 2012; Ogden et al., 2015). There are many complex factors that interact to cause obesity, but one contributor may be daily life stressors. Approximately 40–70% of people report eating more when stressed (Weinstein et al., 1997; Oliver and Wardle, 1999; Epel et al., 2004), and the types of food typically chosen are highly palatable, calorically dense foods (Oliver and Wardle, 1999; Epel et al., 2001; Cartwright et al., 2003; Zellner et al., 2006, 2007; Laugero et al., 2011; Groesz et al., 2012; Kim et al., 2013). People may select these foods for their ability to reduce negative emotions or stressful feelings, a concept often thought of as 'comfort feeding.' Indeed, literature reports show that palatable food can decrease psychological and physiological measures of stress in people (Anderson et al., 1987; Markus et al., 2000; Dubé et al., 2005; Gibson, 2006; Macht and Mueller, 2007; Tomiyama et al., 2011; Tryon et al., 2013). Studies utilizing rodent models have also shown similar effects. During chronic stress, rodents preferentially shift their intake to more highly palatable foods when given a choice (Minor and Saade, 1997; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Packard et al., 2014), and a history of palatable food ingestion can reduce stress responses in rodents, including activation of the neuroendocrine hypothalamic–pituitary–adrenocortical (HPA) axis (Bell et al., 2002; Dallman et al., 2003; la Fleur et al.,

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Abbreviations: BLA, basolateral amygdala; CeA, central amygdala; HPA, hypothalamic–pituitary–adrenocortical; KPBS, potassium phosphate-buffered saline; LSI, Limited sucrose intake; NAc, nucleus accumbens; PBS, phosphate-buffered saline; pCREB, phospho-cyclic AMP response element binding protein; PFC, prefrontal cortex; PVN, paraventricular nucleus of the hypothalamus; RIA, radioimmunoassay.

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2004, 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2007, 2010; Kinzig et al., 2008; Coccurello et al., 2009; Warne, 2009; Finger et al., 2011).

In order to study the neurobiological mechanisms underlying palatable-food mediated stress relief, our group developed a rodent model based on human snacking patterns (Ulrich-Lai et al., 2007, 2010). In this limited sucrose intake (LSI) paradigm, adult, male rats with *ad libitum* access to standard chow and water are also offered a limited amount (up to 4 ml/session; 8 ml/-day) of a 30% sucrose drink (vs. water as a control) twice-daily. Two weeks of LSI reduces HPA axis responses to a subsequent acute restraint stress challenge in male rats. LSI also impacts brain circuits that regulate stress and reward in male rats. For example, LSI increases the mRNA and protein expression of numerous plasticity-related genes in the basolateral amygdala (BLA) in a basal, unstressed state (Ulrich-Lai et al., 2010; Christiansen et al., 2011; Egan and Ulrich-Lai, 2015; Packard et al., 2017). Advanced statistical analyses, including Bayesian modeling, indicate that LSI also modifies the predicted relationships among multiple stress- and reward-regulated brain regions (Ulrich-Lai et al., 2016). Collectively our findings suggest that LSI alters BLA functional connectivity in males.

Importantly, while the effects of LSI are well-characterized in male rats, the effects in female rats are unknown, despite the fact that there may be important sex and estrous cycle differences in stress-dampening by palatable foods. For instance, women may be more prone to emotional or 'comfort feeding' behaviors than men (Grunberg and Straub, 1992; Greeno and Wing, 1994; Oliver and Wardle, 1999; Oliver et al., 2000; Wansink et al., 2003; Klein et al., 2004; Zellner et al., 2006). Emotional eating is also affected by the menstrual cycle, as measured by salivary hormone levels, with the greatest amount of emotional eating during the luteal phase, when estrogen levels are relatively high (Racine et al., 2013; Klump et al., 2013a,b; Hildebrandt et al., 2015). Consistent with this idea, most reward- and feeding-related brain regions express estrogen receptors (ERs) (Shughrue et al., 1997, 1998; Osterlund et al., 1998; Shughrue and Merchenthaler, 2001; Merchenthaler et al., 2004). Likewise, many stress-regulatory brain regions express ER (Shughrue et al., 1997, 1998; Osterlund et al., 1998; Shughrue and Merchenthaler, 2001; Merchenthaler et al., 2004), and HPA responsivity/tone may also be impacted by the estrous cycle (Viau and Meaney, 1991, 2004; Carey et al., 1995; Walker et al., 2001), though not all papers report cycle-related HPA effects (Guo et al., 1994; Bland et al., 2005; Babb et al., 2013). Taking all of these factors into account, we hypothesized that the stress-blunting effects of LSI may differ between female and male rats, and may also be affected by estrous cycle phase.

In order to test this hypothesis, the current study investigated the effects of LSI on HPA axis responses to acute stress, as well as whether these effects vary with estrous cycle stage. The impact of LSI on FosB/deltaFosB- and phospho-cyclic AMP response element binding protein (pCREB)-immunolabeling was later assessed in multiple stress- and reward-regulatory brain regions across the estrous cycle in the basal, unstressed state. This immunolabeling approach was selected for four primary reasons. First, prior experiments in male rats demonstrate that FosB/deltaFosB- and pCREB-immunolabeling are increased by a history of LSI in the basal, unstressed state in several brain regions that regulate stress and reward (Ulrich-Lai et al., 2010, 2016; Christiansen et al., 2011; Egan and Ulrich-Lai, 2015). Second, the expression of these transcription factors can enable the assessment of prolonged effects that accompany the chronic, repeated sucrose intake pattern of the LSI paradigm. For instance, while the phosphorylation of CREB to form activated pCREB occurs rapidly during neuronal activation, pCREB expression can also be prolonged, particularly after chronic, repeated or sustained activation (Bito et al., 1996; Laifenfeld et al., 2005; Rybnikova et al., 2008; Kreibich et al., 2009). Likewise, FosB is a member of the Fos immediate early gene family that is rapidly and transiently expressed following neuronal activation (Hope et al., 1992; Nestler, 2008), while deltaFosB is a truncated form of the full-length FosB that resists degradation and accumulates with chronic or repeated stimulation (Nestler et al., 1999). Consistent with this idea, pCREB and FosB/deltaFosB immunolabeling are increased in the BLA for at least 18 h after the last sucrose exposure in male LSI rats (Ulrich-Lai et al., 2010, 2016; Christiansen et al., 2011). Third, while these transcription factors are well-established markers of neuronal activation, they are also associated with neuroadaptation and/or neural plasticity. pCREB is thought to be critical for long-term potentiation and learning and memory processes (Silva et al., 1998; Huang et al., 2000; Miyamoto, 2006). FosB/deltaFosB expression is also linked with long-term changes in neural plasticity and neuroadaptation, including following chronic treatment with pharmacological rewards like drugs of abuse, and natural rewards like sexual activity and palatable food intake (Chen et al., 1997; Nestler et al., 1999; McClung et al., 2004; Wallace et al., 2008; Vialou et al., 2010; Christiansen et al., 2011; Nestler, 2013). Finally, exploratory Bayesian network analyses can be performed on the immunolabeling data to discover the most likely neural network whose functional relationships are altered by sucrose in an estrous cycle-specific manner in female rats.

Experimental procedures

Animals. Adult, female Long-Evans rats (~175 g body weight, and ~8–10 weeks of age) were acquired from Envigo (formerly Harlan Laboratories, Indianapolis, IN). Rats were individually housed in a temperature- and humidity-controlled facility that is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). Rats were maintained on a 12/12-h light/dark cycle (with lights on at 06:00 h) and acclimated to the housing facility for at least one week before experimental onset. Experimental procedures were approved by the University of

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