NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

S. Zhang et al. / Neuroscience xxx (2018) xxx–xxx

Sex Differences in the Neuroadaptations of Reward-related Circuits in Response to Subchronic Variable Stress

Song Zhang,[a,b,c] Hongxing Zhang,[a,b] Stacy M. Ku,[a,d] Barbara Juarez,[a,d] Carole Morel,[a] Nikos Tzavaras,[e] Sarah Montgomery,[a] Georgia E. Hodes,[f] Anna Brancato,[f] Scott J. Russo,[f] Jun-Li Cao[b*] and Ming-Hu Han[a,f*]

[a] *Department of Pharmacological Sciences and Institute for Systems Biomedicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA*

[b] *Jiangsu Province Key Laboratory of Anesthesiology, Xuzhou Medical University, Xuzhou, Jiangsu 221004, China*

[c] *Department of Anesthesiology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200000, China*

[d] *Neuroscience Program, Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA*

[e] *Microscopy CORE, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA*

[f] *Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA*

Abstract—Women are twice as likely to be diagnosed with major depressive disorder. However, fewer studies in rodent models of depression have used female animals, leading to a relative lack of understanding of the female brain's response to stress, especially at a neural circuit level. In this study, we utilized a 6-day subchronic variable stress (SCVS) mouse model and measured novelty suppressed feeding as behavioral criteria to evaluate susceptibility to SCVS in male and female mice. First, we showed that SCVS induced a decrease in latency to eat (susceptible phenotype) in female mice, but not in males (resilient phenotype). After determining behavioral phenotypes, we investigated the firing activities of dopamine (DA) neurons in the ventral tegmental area (VTA), as well as the neurons that project from lateral habenula (LHb) to the VTA and from locus coeruleus (LC) to the VTA. Utilizing retrograding lumafluor fluorescent tracers and electrophysiology techniques, we performed cell type- and circuit-specific measures of neuronal firing rates. Our data show that SCVS significantly increased the firing rate of LHb-VTA circuit neurons in female mice when compared to that of their female controls, an effect that was absent in SCVS-exposed males. Interestingly, SCVS did not induce significant firing alterations in VTA DA neurons and LC-VTA circuit neurons in either female mice or male mice when compared to their stress-naïve controls. Overall, our data show sex differences in the LHb-VTA circuit responses to SCVS, and implicates a potential role of this projection in mediating vulnerability of female mice to stress-induced depression.

Key words: sex difference, major depression, neuronal activity, ventral tegmental area, lateral habenula, locus coeruleus.

INTRODUCTION

According to a WHO report (Piccinelli and Gomez Homen, 1997), women are twice as likely as men to be diagnosed with stress-related psychiatric disorders, including major depression and anxiety disorder (Kessler et al., 1994; Kendler et al., 1995; Kessler, 2003). It has been suggested that females may respond differently to stress and use distinct stress-coping strategies as compared to males (Kendler et al., 2001; Maciejewski et al., 2001; Klein and Corwin, 2002; Nemeroff et al., 2006). While widely used rodent models of depression have contributed enormously to unravel the neural mechanisms that underlie behavioral responses to stress, these animal models have largely used males only (Solomon et al., 2007; Dalla et al., 2008; Trainor et al., 2011; Ver Hoeve et al., 2013). Due to these limitations, the mechanisms underlying sex differences in stress vulnerability remain largely unknown.

The subchronic variable stress (SCVS) model is used to cause stress-induced depression by exposing mice to three alternating stressors across 6 days (LaPlant et al., 2009; Hodes et al., 2015). Historically, this paradigm induces a depressive phenotype in females, but not in

*Corresponding authors. Address: Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA (M.-H. Han).
E-mail addresses: caojl0310@aliyun.com (J.-L. Cao), ming-hu.han@mssm.edu (M.-H. Han).

Abbreviations: aCSF, artificial cerebrospinal fluid; CMS, chronic mild stress; CRF, corticotropin-releasing factor; DA, dopamine; DBS, deep-brain stimulation; LC, locus coeruleus; LH, learned helplessness; LHb, lateral habenula; MHb, medial habenula; NAc, nucleus accumbens; NE, norepinephrine; NSF, novelty suppressed feeding; RSDS, repeated social defeat stress; SCVS, subchronic variable stress; VTA, ventral tegmental area.

https://doi.org/10.1016/j.neuroscience.2018.02.021
0306-4522/

Please cite this article in press as: Zhang S et al. Sex Differences in the Neuroadaptations of Reward-related Circuits in Response to Subchronic Variable Stress. Neuroscience (2018), https://doi.org/10.1016/j.neuroscience.2018.02.021

males. Thus, the SCVS model provides an ideal model for exploring sex differences of vulnerability to stress. Based on this model, it has also been demonstrated that male and female mice show differential patterns of gene expression in the nucleus accumbens (NAc) (Hodes et al., 2015). Furthermore, Dnmt3a and NF-κB have been identified as important mediators that contribute to sex differences between male and female mice in stress susceptibility (LaPlant et al., 2009; Hodes et al., 2015). In contrast, less is known about the neurophysiological mechanisms that underlie sex differences in stress vulnerability.

The ventral tegmental area (VTA) dopamine (DA) system is a key part of the brain's reward circuitry and plays an important role in mediating stress response (Chaudhury et al., 2013; Friedman et al., 2014). The lateral habenula (LHb) and the locus coeruleus (LC) both send substantial efferents to innervate VTA DA neurons (Omelchenko et al., 2009; Chandler et al., 2013; Juarez and Han, 2016). Accordingly, multiple lines of evidence have demonstrated that the VTA, LHb and LC brain areas all play crucial roles in mediating stress responses. The firing activities of neurons in these three brain regions are altered when exposed to various stressors (Ungless et al., 2004; Krishnan et al., 2007; Sartorius and Henn, 2007; Cao et al., 2010; Li et al., 2011, 2013; Valenti et al., 2012; Isingrini et al., 2016). Furthermore, recent studies show that the firing activities of VTA DA neurons, LHb-VTA circuit neurons and LC-VTA circuit neurons determine susceptible versus resilient behaviors seen in male animals in a variety of stress-induced psychiatric disorders (Li et al., 2011; Chaudhury et al., 2013; Tye et al., 2013; Isingrini et al., 2016). However, how the activity of these neurons is affected following the SCVS paradigm remains unknown in both female and male mice.

Here, utilizing the SCVS model and cell type- and projection-specific *in vitro* electrophysiological recording techniques, we investigated the firing activity alterations of VTA DA neurons, LHb-VTA projecting neurons and LC-VTA projecting neurons in both male and female mice following SCVS. Our findings provide useful evidence that the hyper-activation of the LHb-VTA circuit may play an important role in mediating the vulnerability of female mice to stress-related disorders, as compared to males.

EXPERIMENTAL PROCEDURES

Animals

Seven-week-old C57BL/6J female and male mice (The Jackson Laboratory) were used to set up the SCVS paradigm. All mice were group-housed on a 12-h light/dark cycle with food and water available *ad libitum*. Following the last day of SCVS, all mice were singly housed for behavioral testing and *in vitro* recording. All procedures were approved by the Institutional Animal Care and Use Committee of the Icahn School of Medicine at Mount Sinai and in accordance with the National Institutes of Health guidelines. Twenty-eight female mice and twenty-five male mice were used in this study. Electrophysiological recordings were obtained from the same cohort of mice after behavioral tests (see below).

Subchronic variable stress

SCVS was performed as described previously (Hodes et al., 2015). Female and male mice were put through three unpredictable stressors over 6 days (Fig. 1A). To prevent habituation, mice were subjected to stress in the following order: 100 random foot shocks at 0.45 mA for 1 h (6–8 mice/chamber) on day 1 and day 4; tail suspension stress in which all mice were fixed to hang in an inverted position for 1 h on day 2 and day 5; and restraint stress, in which mice were placed inside a 50-ml falcon tube for 1 h within the home cage on day 3 and day 6. After each stressor, mice were returned to their home cage except on the last day of SCVS when they were then singly housed.

Novelty suppressed feeding (NSF)

It has been shown that the SCVS paradigm induces several consistent behavioral deficits in female mice, but not in males, specifically depression-associated behaviors (Hodes et al., 2015). To minimize the effect of behavioral test-related stress on our electrophysiological recordings, we chose less stressful NSF as a test to confirm the behavioral phenotypes before carrying out the electrophysiological experiments. The NSF test was carried out as previously described (Santarelli et al., 2003; Hodes et al., 2015). Briefly, mice were food deprived 24 h before testing. Water was offered *ad libitum*. On the day of testing, mice were transferred to the testing room 1 h prior to start of the experiment. Under red light conditions, corncob bedding was lightly distributed on the floor of a plastic box of 50 × 50 × 20 cm. A single food pellet was placed on a platform where a petri dish was covered with a white circle cut out from Whatman paper and the platform was positioned in the center of the box. Mice were then placed in the corner of the box and a timer was started. The latency for mice grasping the food pellet with their forepaws and biting was recorded with a limit up to 10 min during testing. As soon as the mice began to eat, or the 10-min time limit was reached, they were immediately transferred back to their home cage.

In vitro slice electrophysiology

The electrophysiological recording procedures were followed as previously described (Chaudhury et al., 2013; Friedman et al., 2014). Under blinded conditions, mice were anesthetized with isoflurane and perfused immediately for 40–60 s with ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 128 NaCl, 3 KCl, 1.25 NaH_2PO_4, 10 D-glucose, 24 $NaHCO_3$, 2 $CaCl_2$ and 2 $MgCl_2$ (oxygenated with 95% O_2 and 5% CO_2, pH 7.4, 295–305 mOsm). Acute brain slices (250 μm) containing VTA, LHb or LC were cut using a vibratome microslicer (DTK-1000, Ted Pella) in ice-cold sucrose aCSF, which was derived by fully replacing NaCl with 254 mM sucrose and saturated by 95% O_2 and 5% CO_2. Slices were maintained in holding chambers with

Please cite this article in press as: Zhang S et al. Sex Differences in the Neuroadaptations of Reward-related Circuits in Response to Subchronic Variable Stress. Neuroscience (2018), https://doi.org/10.1016/j.neuroscience.2018.02.021