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# SUB-CHRONIC VARIABLE STRESS INDUCES SEX-SPECIFIC EFFECTS ON GLUTAMATERGIC SYNAPSES IN THE NUCLEUS ACCUMBENS☆

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**Abstract—Men and women manifest different symptoms of depression and under current diagnostic criteria, depression is twice as prevalent in woman. However, little is known of the mechanisms contributing to these important sex differences. Sub-chronic variable stress (SCVS), a rodent model of depression, induces depression-like behaviors in female mice only, modeling clinical evidence of higher susceptibility to mood disorders in women. Accumulating evidence indicates that altered neuroplasticity of excitatory synapses in the nucleus accumbens (NAc) is a key pathophysiological feature of susceptibility to social stress in males. Here we investigated the effects of SCVS on pre- and post-synaptic protein levels and morphology of glutamatergic synapses of medium spiny neurons (MSNs) in the NAc of female and male mice. Animals underwent six-day exposure to alternating stressors including shock, tail suspension and restraint. MSNs from the NAc were filled with a Lucifer yellow dye and spine density and type were examined using NeuronStudio. In a separate group of animals, immunofluorescence staining was performed for vesicular glutamate transporter 1 (VGLUT1) and vesicular glutamate transporter 2 (VGLUT2), in order to label cortical and subcortical glutamatergic terminals. Immunostaining for post-synaptic density 95 (PSD95) was employed to evaluate post-synaptic density. Females demonstrated circuit-specific pre-synaptic alterations in VGLUT1 and VGLUT2 containing synapses that may contribute to stress susceptibility in the absence of post-synaptic alterations in PSD95 puncta, spine density or type. These data indicate that susceptibility to stress in females is associated with changes in the frequency of distinct glutamatergic inputs to the NAc.** **© 2017 IBRO. Published by Elsevier Ltd. All rights reserved.**

**Key words: stress, sex differences, depression, nucleus accumbens, medium spiny neurons, plasticity.**

## INTRODUCTION

Stress can precipitate or exacerbate depression in "at risk" individuals (Foland-Ross et al., 2014). A variety of rodent stress models have been developed in order to study the neurobiology of stress-induced depression (Menard et al., 2016). However, less attention has been paid to sex differences in stress and depression susceptibility, despite the fact that major depression is twice as common in females as in males (Kessler et al., 1994; Marcus et al., 2005) Important behavioral and neurobiological sex differences exist in most rodent stress models (Dalla et al., 2005, 2008a; Trainor et al., 2011), which provides valuable insight into depression pathophysiology in women.

The subchronic variable stress (SCVS) paradigm involves alternating exposure to different stressors, including foot shock, tail suspension and restraint stress across a six-day period. As a result, female mice display a stress susceptible phenotype in a behavioral battery tailored to model core symptoms of depression (Hodes et al., 2015). In particular, female mice show increased passive coping in the forced swim test, decreased hedonic reactivity in the sucrose preference test, decreased self-grooming in the splash test and higher latency to eat in novelty suppressed feeding, along with no alterations of anxiety-like exploratory behaviors. Importantly, none of these behavioral deficits are observed in male mice following SCVS (LaPlant et al., 2009; Hodes et al., 2015). As such, SCVS allows us to examine which biological changes are correlated with enhanced female stress susceptibility. For example, exposure to SCVS also increases serum corticosterone levels in female mice only and reveals sex-specific alterations of the transcriptome profile in the nucleus accumbens (NAc), a key region of brain reward circuitry (Hodes et al., 2015; Pfau et al., 2016).

Recent evidence implicates the dysregulation of NAc glutamatergic transmission as a key pathophysiological feature of stress and depression susceptibility (Russo

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*Abbreviations:* ILT, intralaminar thalamus; MSNs, medium spiny neurons; NAc, nucleus accumbens; NDS, normal donkey serum; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PSD95, post-synaptic density 95; SCVS, sub-chronic variable stress; TH, tyrosine hydroxylase; VGLUT1, vesicular glutamate transporter 1; VGLUT2, vesicular glutamate transporter 2.

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and Nestler, 2013; Thompson et al., 2015). Direct infusion of glutamate into the NAc dose-dependently decreased swimming time in the forced swim test, whereas intra NAc or systemic injection of NMDA receptor antagonists resulted in antidepressant effects (Rada et al., 2003; Autry et al., 2011). Notably, in males, stress susceptibility is associated with functional and structural neuroplasticity at excitatory synapses in the NAc (Christoffel et al., 2011b). Chronic mild stress and chronic social stress altered AMPAR profile, decreasing GluA2 and increasing GluA1 protein expression in the NAc, thus increasing excitatory synaptic strength (Toth et al., 2008; Vialou et al., 2010). Moreover, chronic mild stress and chronic social stress also act post-synaptically to alter dendritic architecture via changes in spine density and dendritic length in the NAc of males (Christoffel et al., 2011a; Bessa et al., 2013). In particular, chronic social defeat stress resulted in an increase of stubby spines, which negatively correlated with social interaction (Christoffel et al., 2011a), whereas chronic mild stress increased dendritic branching and spine number (Bessa et al., 2013). Furthermore, epigenetic regulation of the synaptic remodeling gene, RAC1, which lead to increased spine density in the NAc of male mice, increased social avoidance and sucrose preference deficits in susceptible mice (Golden et al., 2013). Sex-specific effects of stress on plasticity have been reported for other brain regions, for example acute stress increased spine density in male rats on pyramidal neurons in area CA1 of the hippocampus whereas the same stressor decreased spine density in females (Shors et al., 2001). To date, it is unknown whether stress alters synaptic plasticity mechanisms in the NAc of females.

In addition to the postsynaptic plasticity described above, changes in density of vesicular glutamate transporters (VGLUTs), which mark glutamatergic presynaptic axon terminals (Fremeau et al., 2001), can impact glutamatergic signaling in NAc (Stuber et al., 2010). Among the three VGLUT isoforms, VGLUT1 and VGLUT2 control glutamate vesicle loading and presynaptic release of glutamate. Importantly, they are largely segregated in the brain: VGLUT1 mRNA is primarily found in neurons of the cerebral cortex, hippocampus, basolateral amygdala and cerebellar cortex (Bellocchio et al., 1998; Fremeau et al., 2001, 2004). In contrast, VGLUT2 mRNA is mainly expressed in neurons of the thalamus, brainstem and deep cerebellar nuclei (Fremeau et al., 2001, 2004; Varoqui et al., 2002). In the NAc, which receives input from both VGLUT1-expressing and VGLUT2-expressing neurons, the projections mainly segregate based on input (Hartig et al., 2003). Indeed, because of their different pattern of expression, VGLUT isoforms can serve as pre-synaptic markers to evaluate the neuroplasticity of distinct glutamate inputs to the NAc. Interestingly, the rearrangement of synaptic strength, neuronal processes and axon terminals induced by chronic stress in the NAc of male mice correlated with the susceptible behavioral phenotype, while opposite changes or no differences were observed in resilient animals with respect to unstressed controls (Vialou et al., 2010; Christoffel et al., 2011b, 2015).

In the current study we characterized pre and post-synaptic plasticity of excitatory synapses in the NAc in order to elucidate potential mechanisms of sex-specific stress susceptibility. In particular, we focused on the NAc shell subregion, which is considered a part of the extended amygdala and is primarily involved in the control of motivation and reward. Indeed, in the NAc shell a high degree of convergence of monosynaptic glutamatergic inputs was reported onto individual medium spiny neurons (MSNs) (O'Donnell and Grace, 1993; Mulder et al., 1998). Furthermore, stress and stress hormones exert shell-specific effects (Kalivas and Duffy, 1995; Barrot et al., 1999; Campioni et al., 2009).

We evaluated the immunofluorescence for VGLUT1 and VGLUT2, as pre-synaptic markers of distinct glutamatergic inputs to the NAc. The VGLUT immunofluorescence represents glutamate transporting vesicles predominately located in glutamatergic terminals (Fujiyama et al., 2001). However, the expression of VGLUT2 has been reported in a subset of dopaminergic terminals from the VTA (Stuber et al., 2010). Thus, the levels of co-localization between VGLUT2 and the catecholamine biosynthetic enzyme tyrosine hydroxylase (TH) was also assessed to further characterize the neurochemical nature of NAc VGLUT2-positive puncta(Mendez et al., 2008). To examine post-synaptic plasticity, we filled MSNs with a Lucifer yellow fluorescent dye and used the semi-automated program Neuron Studio to quantify spine density and examine spine morphology (Radley et al., 2008; Dumitriu et al., 2011). We further validated our post-synaptic data by examining the frequency of post-synaptic density 95 (PSD95) puncta, a protein enriched in the post-synaptic density.

## EXPERIMENTAL PROCEDURES

### Animals

C57BL/6 J female and male mice (Jackson Laboratory, Bar Harbor, ME, USA) were used at 8 weeks of age. Mice were group housed and maintained on a 12-h light/dark cycle with *ad libitum* access to food and water. Procedures were performed in accordance with the Institutional Animal Care and Use Committee guidelines of the Icahn School of Medicine at Mount. Sinai.

### Subchronic variable stress

SCVS was performed as described previously (LaPlant et al., 2009; Hodes et al., 2015). Female and male mice ($n$ = 5–6 per group) underwent 1-h of variable stress each day for six days, consisting of foot shock, tail suspension and restraint. To prevent habituation, stressors were administered in the following order (Fig. 1a): 100 random mild foot shocks at 0.45 mA for 1 h (Med Associates, St. Albans, Vermont, USA) (10 mice at the same time in the chamber); tail suspension stress for 1 h; restraint stress – mice were placed inside a 50-ml falcon tube for 1 h within the home cage. The three stressors were then repeated for the next three days in the same

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