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Metabolomics reveals distinct neurochemical profiles associated with stress resilience

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ABSTRACT

Acute social defeat represents a naturalistic form of conditioned fear and is an excellent model in which to investigate the biological basis of stress resilience. While there is growing interest in identifying biomarkers of stress resilience, until recently, it has not been feasible to associate levels of large numbers of neurochemicals and metabolites to stress-related phenotypes. The objective of the present study was to use an untargeted metabolomics approach to identify known and unknown neurochemicals in select brain regions that distinguish susceptible and resistant individuals in two rodent models of acute social defeat. In the first experiment, male mice were first phenotyped as resistant or susceptible. Then, mice were subjected to acute social defeat, and tissues were immediately collected from the ventromedial prefrontal cortex (vmPFC), basolateral/central amygdala (BLA/CeA), nucleus accumbens (NAc), and dorsal hippocampus (dHPC). Ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UPLC-HRMS) was used for the detection of water-soluble neurochemicals. In the second experiment, male Syrian hamsters were paired in daily agonistic encounters for 2 weeks, during which they formed stable dominant-subordinate relationships. Then, 24 h after the last dominance encounter, animals were exposed to acute social defeat stress. Immediately after social defeat, tissue was collected from the vmPFC, BLA/CeA, NAc, and dHPC for analysis using UPLC-HRMS. Although no single biomarker characterized stress-related phenotypes in both species, commonalities were found. For instance, in both model systems, animals resistant to social defeat stress also show increased concentration of molecules to protect against oxidative stress in the NAc and vmPFC. Additionally, in both mice and hamsters, unidentified spectral features were preliminarily annotated as potential targets for future experiments. Overall, these findings suggest that a metabolomics approach can identify functional groups of neurochemicals that may serve as novel targets for the diagnosis, treatment, or prevention of stress-related mental illness.

1. Introduction

Stress is a contributing factor in the etiology of several psychiatric conditions including depression (Heim et al., 2008), panic disorder (Abelson et al., 2007), and post-traumatic stress disorder (PTSD) (Meewisse et al., 2007). Aggression is a particularly salient form of trauma, and people exposed to interpersonal violence are at a greater risk for developing PTSD than those exposed to non-personal trauma (Charuvastra and Cloitre, 2008). However, many individuals who experience stressful events do not develop a stress-related psychopathology, and there is a great deal of interest in what makes certain individuals resilient. Stress resilience refers to the ability of individuals to maintain normal levels of psychological, biological, and social functioning following a traumatic event. Importantly, resilience is an active process and not simply the absence of a pathological response to stress (Charney, 2004; Russo et al., 2012; Feder et al., 2009).

Animal models of social defeat stress have been put forth as high validity models of stress-related mental illness and, interestingly,

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individuals exhibit pronounced variability to the effects of social defeat (Nestler and Hyman, 2010). Genetically identical, inbred mice display a great deal of variability in social avoidance following 10 days of chronic social defeat (Krishnan et al., 2007; Berton et al., 2006) and two days of repeated social defeat (Meduri et al., 2013; Dulka et al., 2015). Susceptible mice avoid novel animals in a social interaction test following social defeat stress, whereas resilient (or resistant) mice investigate novel animals following social defeat stress in a pattern similar to non-defeated controls (Golden et al., 2011). In the chronic social defeat model, brain-derived neurotrophic factor (BDNF) signaling in a neural circuit involving the ventral tegmental area and nucleus accumbens (NAc) is critical for the expression of defeat-induced social avoidance in susceptible animals (Berton et al., 2006). The ventromedial prefrontal cortex (vmPFC) also provides top-down inhibitory control of the NAc and amygdala, which promotes a resistant phenotype after social defeat stress (Vialou et al., 2014). In a mouse model using a single day of acute social defeat, BDNF signaling in the basolateral amygdala (BLA) is necessary for acquisition of defeat-induced social avoidance (Dulka et al., 2016). The development of the susceptible and resistant phenotypes is largely unknown, although the epigenetic changes that underlie stress vulnerability may be linked to environmental influences during pre-natal and post-natal development, including the establishment of early dominance hierarchies (Peaston and Whitelaw, 2006; Wong et al., 2005).

Syrian hamsters are aggressive and territorial animals that exhibit a striking change in behavior following social defeat stress. Following exposure to a single bout of social defeat, male hamsters fail to defend their home territory and instead exhibit submissive and defensive behavior toward novel non-aggressive intruders for up to one month (Huhman et al., 2003). This stress-induced change in agonistic behavior is called the conditioned defeat response and is similar to the defeat-induced social avoidance shown by rats and mice (Kudryavtseva, 1994; Meerlo et al., 1996). The conditioned defeat response in hamsters is an ethologically relevant form of conditioned fear and is regulated by many of the same brain regions, neural circuits, and neurochemicals as conditioned fear. Neurotransmission in the central amygdala (CeA) is critical for the expression of the conditioned defeat response (Jasnow and Huhman, 2001). In the BLA, NMDA receptors, BDNF, and cAMP response element binding (CREB) protein are each necessary for the acquisition of the conditioned defeat response (Day et al., 2011; Jasnow et al., 2005; Taylor et al., 2001). Neurotransmission in several other brain regions is known to modulate the conditioned defeat response, such as the NAc, ventral hippocampus (vHPC), and vmPFC (Gray et al., 2015; Markham et al., 2010; Markham et al., 2012). A great deal of variation exists in the amount of submissive and defensive behavior exhibited by hamsters following social defeat. To investigate vulnerability to the conditioned defeat response, we allowed dyads of hamsters to establish and maintain dominance relationships and then tested dominant and subordinate animals for their conditioned defeat response. We found that dominant hamsters show a reduced conditioned defeat response and increased c-Fos immunoreactivity in the vmPFC compared to subordinate and control animals (Morrison et al., 2011; Morrison et al., 2014). Furthermore, pharmacological blockade of neural activity in the vmPFC reinstated the conditioned defeat response in dominant hamsters but did not alter conditioned defeat in subordinates or controls (Morrison et al., 2013). While a great deal is known about the brain regions and neural circuitry that control the conditioned defeat response, relatively little is known about the neurochemistry within these structures.

There is growing interest in identifying neurochemical biomarkers to aid in the diagnosis, risk assessment, and prevention of stress-related mental illnesses such as PTSD (Yehuda et al., 2013; Zoladz and Diamond, 2013; Baker, Nievergelt, and O'Connor, 2012). Additionally, neurochemicals identified after a stressor can serve as mechanistic biomarkers, and such biomarkers can be used to improve the treatment of stress-related psychopathologies. While attempts to identify biomarkers continue to be a major focus of biomedical research, at present biomarkers have not made it into clinical application for mental illness. Part of the difficulty is that individual neurochemicals are unlikely to correlate with diagnosis, risk, or treatment response for complex forms of stress-related psychopathology. Taking a multifactorial approach is an essential first step toward developing biomarkers for mental illness. Metabolomics is a quantitative analysis of small molecules present in biological systems and has been increasingly used for the discovery of biomarkers (Griffiths et al., 2007; Oldiges et al., 2007; Kaddurah-Daouk et al., 2008). The use of untargeted metabolomics allows the user to take a discovery-based approach, which initially results in a data generating experiment. After relative quantitation of known metabolites based on pre-determined retention times and accurate mass (<5 ppm), the user is still left with thousands of unidentified spectral features (USFs) that potentially relate to a novel compound.

This study focused on characterizing the neurochemical profiles in select brain regions that distinguish animals that are susceptible and resistant to the effects of acute social defeat stress. In both mice and hamster models, we expected that susceptible and resistant animals would differentially express specific neurochemical metabolites in brain regions known to modulate defeat-induced changes in behavior. Further, a comparative approach is expected to aid in the discovery of biomarkers by identifying similar classes of compounds associated with stress susceptibility and resilience in both animal models.

2. Methods

2.1. Animals and housing conditions

Male C57BL/6 mice (7–8 weeks old, 20–27 g) were used as subjects (Envigo, Indianapolis, IN). Mice were maintained on a 12:12 light/dark cycle with *ad libitum* access to food and water in a temperature controlled room (21 ± 2 °C). Animals were housed in polycarbonate cages (18.4 cm × 29.2 cm x 12.7 cm) with corncob bedding, cotton nesting materials, and wire mesh tops. All behavioral procedures were performed during the first three hours of the dark phase of their cycle. Subjects were handled several times one week prior to social defeat to habituate them to the stress of human handling.

Male Syrian hamsters (3–4 months old, 120–180 g) were obtained from our breeding colony that is derived from animals purchased from Charles River Laboratories (Wilmington, MA). All animals were housed in polycarbonate cages (12 cm × 27 cm × 16 cm) with corncob bedding, cotton nesting materials, and wire mesh tops. Food and water were available *ad libitum*. Cages were not changed for one week prior to dominant–subordinate encounters to allow individuals to scent mark their territory. Subjects were handled several times one week prior to dominant–subordinate encounters to habituate them to the stress of human handling. Animals were housed in a temperature controlled colony room (21 ± 2 °C) and kept on a 14:10 h light:dark cycle to facilitate reproductive maturation. All behavioral protocols were performed during the first 3 h of the dark phase of their cycle. Procedures in both mice and hamsters were approved by the University of Tennessee Institutional Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.