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UNDERSTANDING THE ADDICTION CYCLE: A COMPLEX BIOLOGY WITH DISTINCT CONTRIBUTIONS OF GENOTYPE VS. SEX AT EACH STAGE

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Abstract—Ethanol abuse can lead to addiction, brain damage and premature death. The cycle of alcohol addiction has been described as a composite consisting of three stages: intoxication, withdrawal and craving/abstinence. There is evidence for contributions of both genotype and sex to alcoholism, but an understanding of the biological underpinnings is limited. Utilizing both sexes of genetic animal models with highly divergent alcohol withdrawal severity, Withdrawal Seizure-Resistant (WSR) and Withdrawal Seizure-Prone (WSP) mice, the distinct contributions of genotype/phenotype and of sex during addiction stages on neuroadaptation were characterized. Transcriptional profiling was performed to identify expression changes as a consequence of chronic intoxication in the medial prefrontal cortex. Significant expression differences were identified on a single platform and tracked over a behaviorally relevant time course that covered each stage of alcohol addiction; i.e., after chronic intoxication, during peak withdrawal, and after a defined period of abstinence. Females were more sensitive to ethanol with higher fold expression differences. Bioinformatics showed a strong effect of sex on the data structure of expression profiles during chronic intoxication and at peak withdrawal irrespective of genetic background. However, during abstinence, differences were observed instead between the lines/phenotypes irrespective of sex. Confirmation of identified pathways showed distinct inflammatory signaling following intoxication at peak withdrawal, with a pro-inflammatory phenotype in females but overall suppression of immune signaling in males. Combined, these results suggest that each stage of the addiction cycle is influenced differentially by sex vs. genetic background and support the development of stage- and sex-specific therapies for alcohol withdrawal and the maintenance of sobriety. Published by Elsevier Ltd. on behalf of IBRO.

Key words: prefrontal cortex, ethanol, sexual dimorphism, inflammation, astrocytes, low level response to alcohol.

INTRODUCTION

Alcohol use disorder, a chronic relapsing disease, is a well-recognized public health problem and is one of the leading preventable causes of death worldwide (Rehm et al., 2009). Unfortunately, ethanol is the most common substance of abuse in the US, and abuse can lead to physical dependence and addiction. Alcoholism is a complex disease with multiple risk factors, and there is evidence for both sexual-dimorphism and a genetic contribution in both the risk to develop the disorder and in the detrimental responses that result from alcohol abuse. In general, the alcohol addiction cycle has been described by Koob et al. as consisting of three stages: intoxication, withdrawal and craving/abstinence (Koob and Volkow, 2010). However, understanding of the biological underpinnings of alcohol addiction is limited. In particular, the impact of sex/gender vs. genetic background/phenotype on each stage of the addiction cycle has not been characterized.

Previous reports have demonstrated that sex/gender influences the response to alcohol (Ceylan-Isik et al., 2010). Specific sex differences have been reported in terms of alcohol handling (Addolorato et al., 1999), with body composition differences influencing ethanol partitioning between lipid and water compartments. In addition, females have reduced levels of alcohol dehydrogenase, a key liver enzyme involved in alcohol metabolism and removal (Baraona et al., 2001). Most notably, sex differences have been characterized in numerous alcohol-related behaviors in which females show: reduced risk for the development of ethanol

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Abbreviations: BEC, blood ethanol concentration; Ccl5, chemokine (C-C motif) ligand 5; Ccl11, chemokine (C-C motif) ligand 11; CNS, central nervous system; DAVID, Database for Annotation, Visualization and Integrated Discovery v6.7; GO, Gene Ontology; GSEA, Gene Set Enrichment Analysis; IL, interleukin; IPA, Ingenuity Pathway Analysis; LPS, lipopolysaccharide; Low LR, low level of response; MAPK, mitogen-activated protein kinase; mPFC, medial prefrontal cortex; NF-κB, nuclear factor kappa light-chain-enhancer of activated B cells; NLRP3, nod-like receptor family, pyrin domain containing 3; qPCR, quantitative real-time polymerase chain reaction; RANTES, regulated on activation, normal T cell expressed and secreted; S1P, sphingosine-1-phosphate; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor; WGCNA, weighted gene coexpression network analysis; WSP, Withdrawal Seizure-Prone selected line; WSR, Withdrawal Seizure-Resistant selected line.

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dependence and addiction; increased ethanol sensitivity; increased ethanol consumption; reduced withdrawal severity; and increased risk of recidivism and relapse (Brady and Randall, 1999; Devaud and Chadda, 2001; Sershen et al., 2002; Wang et al., 2003; Carroll et al., 2004; Prescott et al., 2005; Becker and Hu, 2008; Potts et al., 2013). Alcohol may also be more rewarding in females (Blanchard et al., 1993; Torres et al., 2014). Combined, such data stress the importance of sex-specific analysis and place a particular emphasis on improved understanding of mechanisms underlying such responses in female alcoholics (see Wiren, 2013).

In addition to sex differences, the genetic contribution to the overall development of alcoholism is well described (Magnusson et al., 2010). However, understanding of the risks associated with specific polymorphisms/mutations or biological pathways remains quite limited (for review see Han et al., 2013). Given that alcoholism is a disorder of complex genetics with multiple genetic loci that are influenced by interactions with the environment, dissecting genetic contributions to the disease in diverse human populations has proven difficult. Thus, various animal models have been developed that show increased or decreased sensitivity to ethanol as phenotypes. The use of selectively bred rodent lines provides a genetically rich model where the various alleles present in the initial heterogeneous population related to the selection phenotype become differentially segregated with selection pressure in the respective lines. Thus, differences observed in selected lines provide evidence of the genetic underpinnings that influence the trait of interest. Our studies have employed lines of mice with highly divergent withdrawal severity after chronic intoxication derived by selective breeding from heterogeneous stock; the low response to alcohol withdrawal Withdrawal-Seizure Resistant (WSR) and high response Withdrawal-Seizure Prone (WSP) mouse lines (Kosobud and Crabbe, 1986). Evidence of physical dependence is considered a hallmark of alcoholism; one measure of physical dependence is increased neuronal excitability including seizures. Such hyperexcitability during withdrawal is thought to reflect neuroadapations that occur with chronic ethanol intoxication, including changes in gene expression and brain structure which enable an organism to function in the presence of this central nervous system (CNS) depressant. Thus, the WSR and WSP lines are of interest because the large differences in response to chronic alcohol, as evidenced by divergent withdrawal severity, are believed to reflect distinct neuroadaptive responses between the phenotypes that occur with chronic intoxication. Furthermore, WSR and WSP lines also demonstrate sex differences similar to humans since females consume more alcohol yet exhibit reduced withdrawal severity in these lines (Kosobud and Crabbe, 1986).

Current treatments for alcohol dependence are at best only modestly effective (Olive, 2010; Zindel and Kranzler, 2014). Since individuals with alcohol dependence represent a clinically heterogeneous and genetically diverse population, it has been proposed that effective treatments should target specific phenotypes at distinct stages of addiction rather than employ a generic approach to all patients (Kuehn, 2009). Although sex and genotype both contribute to risks of dependence and addiction, the distinct contribution that each may subserve during the stages of the addiction cycle remains uncharacterized as no systematic analyses of the impact of sex vs. genotype on neuroadaptive responses during the addiction cycle following chronic intoxication have been reported. In this work, the WSR and WSP selected lines of mice were employed as preclinical models of genomically rich widely divergent ‘‘response to alcohol’’ phenotypes and both sexes were examined. Analysis was done using tissue from the medial prefrontal cortex (mPFC). In addition to involvement in the addition cycle (Koob and Volkow, 2010), the mPFC participates in hyperexcitability circuitry during withdrawal from chronic exposure (Chen et al., 2009), is important in executive function and inhibitory control (Fuster, 2002; Kroener et al., 2012), and is associated with cognitive dysfunction and damage in alcoholics (Zahr et al., 2011). Analysis was performed at biologically relevant endpoints during the addiction cycle, i.e., after chronic exposure, during peak withdrawal and after a defined period of abstinence following chronic ethanol intoxication, to identify important contributors in the neuroadaptive response. For this comprehensive addiction stage analysis, we employed the same animal models, the same chronic ethanol exposure paradigm and the same array platform, to compare and contrast expression differences over an addiction time course in a defined system. Results demonstrate for the first time that sex and genotype/phenotype have distinct and varying influences on neuroadaptation and result in divergent biological response pathways during each stage of the addiction cycle.

EXPERIMENTAL PROCEDURES

Animal subjects

Two independently derived replicate WSP and WSR lines (Kosobud and Crabbe, 1986) were generated by selective breeding for divergent withdrawal severity from genetically heterogeneous HS/Ibg mice. Female and male mice from both replicates (i.e., WSR-1, -2 and WSP-1, -2) were tested for expression differences. To identify phenotype-specific differences, expression analysis was collapsed on replicate for each selected line. As these lines are employed to identify genetic underpinnings of the selected phenotype, comparisons between the WSR and WSP mice are referred to as either phenotype, genotype or line differences. Mice were maintained under a light/dark cycle of 0600–1800 light with water and Purina Lab Diet chow available *ad libitum*. Room temperatures were maintained at 22 ± 1 °C. Ethanol (20%, v/v) was mixed with 0.9% saline and injected intraperitoneally (i.p.) or introduced without mixing as a vapor into the chambers. All animal procedures were carried out in accordance with the US National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). All procedures were approved by the Portland Oregon VA Medical Center Institutional Animal Care and Use Committee, which mandates that all efforts are made to minimize