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ALTERED METABOLIC ACTIVITY IN THE DEVELOPING BRAIN OF RATS PREDISPOSED TO HIGH VERSUS LOW DEPRESSION-LIKE BEHAVIOR

C. R. MCCOY,[a†] S. R. GOLF,[a†] M. MELENDEZ-FERRO,[b] E. PEREZ-COSTAS,[c] M. E. GLOVER,[a] N. L. JACKSON,[a] S. A. STRINGFELLOW,[a] P. C. PUGH,[a] A. D. FANT[d] AND S. M. CLINTON[a]*

[a] *Department of Psychiatry and Behavioral Neurobiology, 1720 7th Avenue South, SC 745, University of Alabama-Birmingham, AL, USA*

[b] *Department of Surgery, 1600 7th Avenue South, ACC300, University of Alabama-Birmingham, AL, USA*

[c] *Department of Pediatrics, 1600 7th Avenue South, ACC502, University of Alabama-Birmingham, AL, USA*

[d] *Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, 27599, USA*

Abstract—Individual differences in human temperament can increase the risk of psychiatric disorders like depression and anxiety. Our laboratory utilized a rat model of temperamental differences to assess neurodevelopmental factors underlying emotional behavior differences. Rats selectively bred for low novelty exploration (Low Responders, LR) display high levels of anxiety- and depression-like behavior compared to High Novelty Responder (HR) rats. Using transcriptome profiling, the present study uncovered vast gene expression differences in the early postnatal HR versus LR limbic brain, including changes in genes involved in cellular metabolism. These data led us to hypothesize that rats prone to high (versus low) anxiety/depression-like behavior exhibit distinct patterns of brain metabolism during the first weeks of life, which may reflect disparate patterns of synaptogenesis and brain circuit development. Thus, in a second experiment we examined activity of cytochrome C oxidase (COX), an enzyme responsible for ATP production and a correlate of metabolic activity, to explore functional energetic differences in the HR/LR early postnatal brain. We found that HR rats display higher COX activity in the amygdala and specific hippocampal subregions compared to LRs during the first 2 weeks of life. Correlational analysis examining COX levels across several brain regions and multiple early postnatal time points suggested desynchronization in the developmental timeline of the limbic HR versus LR brain during the first two postnatal weeks. These early divergent COX activity levels may reflect altered circuitry or synaptic activity in the early postnatal HR/LR brain, which could contribute to the emergence of their distinct behavioral phenotypes.

Key words: anxiety, neurodevelopment, transcriptome, metabolism, amygdala, cytochrome C oxidase.

INTRODUCTION

Individual differences in temperament and emotional reactivity shape the ability of humans to cope with stress and predispose certain individuals to psychiatric disorders such as depression and anxiety (Cloninger et al., 2006). Our laboratory uses a rodent model of temperamental differences to study the neurobiological basis of differences in emotional behavior. Sprague–Dawley rats that were selectively bred to display low behavioral response to novelty (Low Responders, LR) also exhibit high anxiety- and depression-like behavior compared to High Novelty Responder rats (HRs). These behaviors include diminished sociability and sexual motivation, increased behavioral inhibition and helplessness, as well as greater vulnerability to chronic stress (Stead et al., 2006a; Stedenfeld et al., 2011; Cummings et al., 2013; Clinton et al., 2014; Cohen et al., 2015). Furthermore, these behavioral traits emerge in early life, and a previous microarray study from our group revealed widespread gene expression differences in the early postnatal HR versus LR brain (Clinton et al., 2011). More specifically, numerous genes were altered in the developing hippocampus (HPC) of HR versus LR rats during the first few weeks of life (i.e. postnatal days (Ps) 7, 14 and 21), with marked changes in genes involved in synaptogenesis and cellular metabolism (Clinton et al., 2011).

While dysregulated monoamine neurotransmission has long been hypothesized to underlie the pathophysiology of depression (Hirschfeld, 2000), data also point to a possible role for metabolic dysfunction in the illness (Gardner and Boles, 2011). Numerous human neuroimaging studies have documented abnormal metabolism within several limbic brain regions of individuals suffering from major depression (Fitzgerald et al., 2008;

*Corresponding author. Address: 1720 7th Avenue South, SC 745, Birmingham, AL 35294, USA. Tel: +1-205-975-0312.
E-mail address: clintons@uab.edu (S. M. Clinton).

† Authors contributed equally to the work presented in this manuscript.

Abbreviations: AMY, amygdala; ANOVA, analysis of variance; CA, cornu ammonis; COX, cytochrome C oxidase; DG, dentate gyrus; ds-cDNA, double-stranded cDNA; GEO, gene expression omnibus; GO, gene ontology; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPC, hippocampus; HR, High Novelty Responder rat; LR, Low Responders; P, postnatal day; PB, phosphate buffer; PCA, principal components analysis; PFC, prefrontal cortex.

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Anand et al., 2009), and studies using postmortem brain tissue from depressed patients have reported alterations in genes involved in a variety of metabolic processes (Sequeira et al., 2007; Kim and Webster, 2010; Regenold et al., 2012). Depression and other affective disorders are highly co-morbid with metabolic disorders, and the two classes of illness share many symptoms such as fatigue, cognitive deficits, and psychomotor retardation (Gardner and Boles, 2011). A recent study even showed that monoaminergic (specifically serotonin) and mitochondrial dysfunctions may be linked through monoamine-dependent regulation of phosphorylated glucocorticoid receptors and transcriptional control of mitochondrial genes for cytochrome C oxidase (COX) (Adzic et al., 2013). Whether these processes are overlapping or provide separate mechanisms of action, it is important to gain a deeper understanding of their roles in shaping emotional behavior.

The high energy demands of the brain are predominately fulfilled via oxidative phosphorylation (Hall et al., 2012), and perturbation of this pathway may contribute to metabolic abnormalities known to occur in emotional disorders. ATP is critical for myriad cellular processes in the brain (e.g., neurotransmission, intracellular signaling, calcium buffering, synaptic plasticity) (Babcock and Hille, 1998; Brodin et al., 1999; Li et al., 2004); its synthesis requires proper functioning of the electron transport chain, which consists of four enzyme complexes positioned within the inner membrane of mitochondria (Saddar et al., 2008). COX is the terminal rate-limiting enzyme in the electron transport chain; COX's activity in the brain is directly proportional to ATP production and is commonly used to compare metabolic activity within different regions of the brain in human postmortem studies as well as studies using animal models of psychopathology (Harro et al., 2011; Wong-Riley, 2012; Rice et al., 2014).

Our previous work suggested that HR/LR differences in anxiety/depression-like behavior stem from disparate developmental trajectories in the early postnatal HPC and possibly amygdala (AMY) (Clinton et al., 2011; Cohen et al., 2015). These early-life HR/LR gene expression differences involved several functional classes of genes, including those relevant to synaptogenesis, synaptic plasticity, and cellular metabolism. The present study builds on that work, examining transcript and metabolic changes in the developing LR versus HR limbic brain that may contribute to a high anxiety/depression-like phenotype. We hypothesized that rats prone to high (versus low) levels of anxiety/depression-like behavior would exhibit distinct patterns of gene expression and metabolic activity in the early postnatal limbic brain. We first used transcriptome profiling to assess widespread gene expression in the brains of early postnatal HR/LR pups (Ps 7, 14 and 21), focusing on key brain areas known to regulate emotional behavior: the AMY, HPC and prefrontal cortex (PFC). A second experiment utilized COX histochemistry to map metabolic activity patterns in the developing HR/LR brain to test the hypothesis that developing anxiety/depression-prone LR rats exhibit altered COX activity in one or more brain regions compared to HR pups.

EXPERIMENTAL PROCEDURES

All experiments were approved by the Committee on the Use and Care of Animals at the University of Alabama at Birmingham. This work was performed in accordance with the National Institutes of Health (USA, 2011) and National Research Council (UK, 1996) guidelines on animal research.

Animals and tissue collection

The animals used in this study were obtained from our in-house colony where the HR/LR bred lines were recently re-derived using a breeding strategy described previously in our original publication with the bred HR/LR rats (Stead et al., 2006a). Briefly, we screened 60 male and 60 female Sprague–Dawley rats purchased from Charles River Laboratory based on locomotor activity in a novel environment. Rats were individually placed into Noldus PhenoTyper® Boxes (45 × 45 × 60-cm clear Plexiglas chambers) equipped with video cameras and Ethovision® XT 8.0 software to monitor exploratory behavior (distance traveled) during a 30-min test session. Males and females with the highest and lowest exploration scores were bred together to generate the first generation of HR and LR lines, respectively. When progeny from each generation reached adulthood, we repeated the novelty-induced locomotion test and selected the top- and bottom-scoring animals from HR and LR families, respectively, for subsequent breeding. The present experiments were conducted using tissue from HR/LR males from the 4th generation of our colony. All housing and testing facilities were maintained at 21–23 °C and 50–55% humidity. For all experiments, rats were pair-housed in a 12:12-h light–dark cycle (lights on/off at 6 AM/6 PM).

Adult male/female pairs were mated to produce HR and LR litters. At birth, litters were culled to six male and six female pups to control for litter size and gender composition. HR and LR male offspring were sacrificed by rapid decapitation to harvest brains at three early postnatal time points: P7, P14 and P21 (n = 15/phenotype/time point). Brains were removed, flash frozen in isopentane cooled to −30 °C on dry ice, and then stored at −80 °C until further use. A subset of brains (n = 5/phenotype/time point, with each of the five rats per time point derived from an independent litter) were sectioned on a cryostat at −10 to −12 °C, and alternating sections of 20 and 300 μm were collected. The 20-μm sections were stained with Cresyl Violet and compared to developing and adult rat brain atlases (Paxinos and Watson, 1986; Paxinos et al., 1994) to identify target anatomical regions in the 300-μm sections. Portions of the HPC, AMY, and PFC were removed from the 300-μm-thick sections using a 0.5-mm tissue punch (Harris Micro-Punch, Ted Pella, Redding, CA, USA), stored at −20 °C, and later homogenized. RNA was isolated (NucleoSpin RNA II), quantified using a Nanodrop ND-1000 (Wilmington, DE), and stored at −80 °C. The remaining brains (n = 10/phenotype/time point) were sectioned on a cryostat at 15 μm, mounted onto slides