Neuroscience 264 (2014) 88–98

SOCIAL DEFEAT INDUCES CHANGES IN HISTONE ACETYLATION AND EXPRESSION OF HISTONE MODIFYING ENZYMES IN THE VENTRAL HIPPOCAMPUS, PREFRONTAL CORTEX, AND DORSAL RAPHE NUCLEUS

C. A. KENWORTHY,[a] A. SENGUPTA,[b] S. M. LUZ,[b] E. S. VER HOEVE,[b] K. MEDA,[a] S. BHATNAGAR[b] AND T. ABEL[a]*

[a] *University of Pennsylvania, Philadelphia, PA, USA*

[b] *Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, Philadelphia, PA, USA*

Abstract—Chronic exposure to stress is associated with a number of psychiatric disorders, but little is known about the epigenetic mechanisms that underlie the stress response or resilience to chronic stress. We investigated histone acetylation in seven different brain regions of rats exposed to chronic social defeat stress: the dorsal hippocampus (dHPC), ventral hippocampus (vHPC), medial prefrontal cortex (mPFC), basolateral amygdala (BLA), locus coeruleus (LC), paraventricular thalamus (PVT), and dorsal raphe (DR) nucleus. This stress paradigm was unique in that it allowed rats to display resilience in the form of an active coping mechanism. We found that there was an increase in acetylation of H3K9/14 (H3K9/14ac) and bulk acetylation of H4K5,8,12,16 (H4K5,8,12,16ac) in the DR nucleus of rats that were less resilient. Less resilient rats also displayed increased levels of H3K18 acetylation (H3K18ac) in the mPFC when compared to non-stressed controls. In the vHPC, there was an increase in H3K18ac and H4K12 (H4K12ac) in rats that were less resilient when compared to non-stressed control rats. In addition, there was a decrease in levels of H4K8 acetylation (H4K8ac) in both resilient and non-resilient rats as compared to controls. We assessed expression of histone modifying enzymes in the vHPC and the mPFC using quantitative real-time polymerase chain reaction (PCR) and found changes in expression of a number of targets. These included changes in Sirt1 and Sirt2 in the vHPC and changes in Kat5 in the mPFC. Overall, these results suggest that changes in histone acetylation and expression of histone modifying enzymes in these regions correlate with the behavioral response to stress in socially defeated rats.

*Corresponding author. Address: Department of Biology, University of Pennsylvania, Translational Research Center, Room 10-133, 3400 Civic Center Boulevard, Building 421, Philadelphia, PA 19104-5158, USA. Tel: +1-215-898-3100.

E-mail address: abele@sas.upenn.edu (T. Abel).

Abbreviations: ANOVA, analysis of variance; HATs, histone acetyltransferases; LL, long latency; PCR, polymerase chain reaction; SL, short latency.

This article is part of a Special Issue entitled: Epigenetics in Brain Function.

Key words: epigenetics, chronic stress, resilience, dorsal raphe nucleus, hippocampus, medial prefrontal cortex.

INTRODUCTION

Chronic exposure to stress is associated with a number of psychiatric disorders including depression, generalized anxiety disorder and post-traumatic stress disorder (PTSD), as well as decreased executive function, and decreased cognitive performance (Ehlert et al., 2001; Yehuda, 2001; Nestler et al., 2002; Tsankova et al., 2006). Chronic stress impacts the function of a number of brain regions. These regions include the dorsal raphe (DR) nucleus, which is a major source of serotonergic input to the mammalian forebrain and which has been implicated in the physiological and behavioral response to stress (Azmitia and Segal, 1978; Mamounas et al., 1991; Jacobs and Azmitia, 1992; Petrov et al., 1992; Maier et al., 1993; Lowry, 2002; Lowry et al., 2005). In addition, evidence suggests that chronic stress leads to affective disorders and impairs learning and memory both in humans and in laboratory animals, thus implicating the hippocampus in response to stress (Sapolsky, 2000; McEwen et al., 2002; Sheline et al., 2003; Duman, 2004; Dagyte et al., 2011). Finally, the medial prefrontal cortex (mPFC) has long been of interest due to the role that this region plays in depression and in moderating the behavioral and physiological response to chronic stress (Amat et al., 2005; Cerqueira et al., 2007; Dagyte et al., 2011; Cabib and Puglisi-Allegra, 2012).

Evidence suggests that stress induces changes in gene transcription in these regions (Datson et al., 2012; Donner et al., 2012). Epigenetics is the means through which the environment can lead to lasting and heritable changes in gene transcription. As a result, epigenetic mechanisms are a potentially important underlying cause of numerous psychiatric disease states and may mediate the impact of stress on the function of neural circuits (Tsankova et al., 2006; Sananbenesi and Fischer, 2009; Nelson and Monteggia, 2011).

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.01.024

The underlying mechanisms behind epigenetic changes are various modifications that remodel chromatin, the complex of DNA, histones and non-histone proteins. Of these changes, one of the most important is histone acetylation (Peixoto and Abel, 2012), a post-translational modification of lysine residues that lie on the histone amino terminal tails (Levenson and Sweatt, 2005; Sananbenesi and Fischer, 2009; Morris et al., 2010). This modification is mostly associated with increases in levels of gene transcription (Chuang et al., 2009; Sananbenesi and Fischer, 2009; Morris et al., 2010; Lubin et al., 2011; Trollope et al., 2012).

In this study, we examined the effects of a chronic model of social defeat stress on histone acetylation and the expression of histone modifying enzymes. We had previously shown that rats that exhibit an active coping behavioral phenotype during 7 days of daily defeat are resilient to the effects of defeat whereas those rats that exhibit a passive coping phenotype are vulnerable to the effects of defeat (Wood et al., 2010). In this study, we examined epigenetic changes in brain regions important for regulating neuroendocrine, behavioral, and cognitive responses to stress, including the dorsal hippocampus (dHPC), the ventral hippocampus (vHPC), mPFC, basolateral amygdala (BLA), locus coeruleus (LC), paraventricular thalamus (PVT), and DR nucleus. We found that chronic social defeat stress induced significant changes in histone acetylation in the vHPC, the mPFC, and the DR. We also found that chronic social defeat stress induced significant changes in the expression of a number of genes encoding molecular components that regulate epigenetic modifications in the mPFC and the vHPC. Importantly, these effects of social defeat differed between resilient and vulnerable rats. Together, our findings suggest that vulnerability or resilience to chronic stress alters different epigenetic states in specific regions of the rat brain.

EXPERIMENTAL PROCEDURES

Social defeat stress

Sprague–Dawley rats were subjected to social defeat stress according to the resident-intruder model based on our modifications (Bhatnagar and Vining, 2003; Bhatnagar et al., 2006) of the original paradigm developed by Miczek (1979). Male Sprague–Dawley rats (275–300 g on the first day of experimentation) were the intruders or controls, and male Long–Evans retired breeders (650–850 g) served as residents (Charles River, Wilmington, MA, USA). Rats were singly housed with a 12-h light, 12-h dark cycle (lights on at 07:00 h) in a climate-controlled room with *ad libitum* access to food and water. Experimental intruder rats are placed in the cage of the resident for a total of 30 min per day for 7 consecutive days. If an intruder rat exhibited the supine position for at least 3 s (Bhatnagar and Vining, 2003; Bhatnagar et al., 2006), this was defined as a defeat posture and rats were separated by a wire mesh barrier for the remainder of the 30-min session. If a rat did not exhibit defeat, a wire mesh barrier was placed after 15 min to separate the intruder and resident rat. On each of the 7 days, the intruder rat was exposed to a different resident rat which had been pre-screened for aggression. As we previously noted, over these 7 days, a bimodal distribution formed with some rats exhibiting long latencies to be defeated (LL rats; greater than 300 s averaged over the 7 days) and other rats exhibiting short latencies to be defeated (SL rats; less than 300 s averaged over the 7 days)(Wood et al., 2010). LL rats exhibited increased incidence of boxing and upright postures indicating an active behavioral phenotype whereas the SL rats submitted rapidly to the resident, indicating a passive behavioral phenotype. In addition, a control (C) group was handled and introduced into a novel cage behind a wire partition for 30 min. All experiments were carried out in accordance with National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania and the Children's Hospital of Philadelphia.

Tissue extraction

24 h after the final defeat session, rats were sacrificed through rapid decapitation. The vHPC, dHPC, mPFC, DR nucleus, PVT, BLA, PVT, and LC were rapidly dissected at 4 °C before being snap frozen on dry ice and stored at −80 °C.

Protein and RNA extraction

Protein and RNA were jointly extracted from the dHPC, vHPC, mPFC, BLA, LC, PVT, and DR nucleus by adapting the Qiagen RNeasy extraction method (Qiagen, Valencia, CA, USA). Briefly, cell lysates in buffer RLT (Qiagen, Valencia, CA, USA) were centrifuged through RNeasy spin columns to capture RNA. The flowthrough from these samples then had four volumes of acetone at −20 °C added to them and were incubated at −20 °C for 30 min. These samples were then centrifuged at 4500 relative centrifugal force (rcf) before acetone was drained from the samples and the remaining protein pellet was resuspended in 9 M Urea. RNA was purified and extracted using the standard Qiagen RNeasy (Qiagen, Valencia, CA, USA) RNA extraction protocol and subjected to a Qiagen on-column DNase treatment (Qiagen, Valencia, CA, USA) before final elution.

RNA extraction from the mPFC

The mPFC was freshly dissected from brains of rats sacrificed 24 h after the final stress/control exposure, immediately frozen in dry ice, and stored at −80 °C until further processing. These tissue punches were homogenized in QIAzol Lysis Buffer (Qiagen, Valencia, CA, USA), and total RNA was extracted using the miRNEasy kit with on-column DNase I digestion (Qiagen, Valencia, CA, USA) according to the manufacturer's guidelines. The concentration of RNA in the samples was measured using a Nanodrop ND-1000 spectrophotometer (NanoDrop products, Wilmington, DE, USA).

Histone acetylation

Histone acetylation was examined through Western blotting of protein precipitated out of buffer RLT. 10 μg of sample was loaded into a 4–12% bis–tris gel. After affecting separation, protein was transferred to a polyvinylidene fluoride (PVDF) membrane and immunoblotted for histone residues H3K18ac (Cell Signaling; Cat. No. 9675), H3K9/14ac (Millipore; Cat. No. 04-1003), H4K5,8,12,16ac (Millipore; Cat No. 12-353), H4K8ac (Cell Signaling; Cat No. 2594), and H4K12ac (Cell Signaling; Cat. No. 2591). All results were normalized to beta tubulin (Sigma–Aldrich; Cat. No. T4026).