Neuroscience 246 (2013) 142–159

INDIVIDUAL DIFFERENCES IN THE EFFECTS OF CHRONIC STRESS ON MEMORY: BEHAVIORAL AND NEUROCHEMICAL CORRELATES OF RESILIENCY

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Abstract—Chronic stress has been shown to impair memory, however, the extent to which memory can be impaired is often variable across individuals. Predisposed differences in particular traits, such as anxiety, may reveal underlying neurobiological mechanisms that could be driving individual differences in sensitivity to stress and, thus, stress resiliency. Such pre-morbid characteristics may serve as early indicators of susceptibility to stress. Neuropeptide Y (NPY) and enkephalin (ENK) are neurochemical messengers of interest implicated in modulating anxiety and motivation circuitry; however, little is known about how these neuropeptides interact with stress resiliency and memory. In this experiment, adult male rats were appetitively trained to locate sugar rewards in a motivation-based spatial memory task before undergoing repeated immobilization stress and then being tested for memory retention. Anxiety-related behaviors, among other characteristics, were monitored longitudinally. Results indicated that stressed animals which showed little to no impairments in memory post-stress (i.e., the more stress-resilient individuals) exhibited lower anxiety levels prior to stress when compared to stressed animals that showed large deficits in memory (i.e., the more stress-susceptible individuals). Interestingly, all stressed animals, regardless of memory change, showed reduced body weight gain as well as thymic involution, suggesting that the effects of stress on metabolism and the immune system were dissociated from the effects of stress on higher cognition, and that stress resiliency seems to be domain-specific rather than a global characteristic within an individual. Neurochemical analyses revealed that NPY in the hypothalamus and amygdala and ENK in the nucleus accumbens were modulated differentially between stress-resilient and stress-susceptible individuals, with elevated expression of these neuropeptides fostering anxiolytic and pro-motivation function, thus driving cognitive resiliency in a domain-specific manner. Findings suggest that such neurochemical markers may be novel targets for pharmacological interventions that can serve to prevent or ameliorate the negative effects of stress on memory.

Key words: stress, memory, resiliency, anxiety, neuropeptide-Y, enkephalin.

INTRODUCTION

Repeated exposure to social and physical stress alters neurochemical pathways in the rat brain associated with motivated behaviors and dopaminergic activity (Lucas et al., 2004, 2007; Suzuki et al., 2010; Yohe et al., 2012). Memory acquisition, specifically hippocampal-dependent spatial memory, has been demonstrated in rats using motivation-based behavioral paradigms in which animals are appetitively motivated to learn the location of a food reward (Olton et al., 1977); and, exposure to stress (both social and physical) has been shown to interfere with motivation and memory (Kleen et al., 2006). Furthermore, in animal models of post-traumatic stress disorder (PTSD), stress-induced affective, metabolic, and immune system disorders are often characterized alongside cognitive impairments (Yehuda and Antelman, 1993; Pynoos et al., 1996). However, individual differences in the extent to which chronic stress can impact the body, brain, and behavior have only more recently been explored.

Individual variation in stress responses and their long-term effects are of clinical significance and several investigators have examined how individual differences in pre-morbid behavior, e.g., locomotor reactivity to a novel environment or emotion–cognition interactions, could predict and explain variability in the effects of stress on memory and its underlying neural mechanisms (Sandi et al., 2004; Touyarot et al., 2004; Antoniou et al., 2008; Sandi, 2008). Individuals which had higher locomotor reactivity in response to a mild stressor (i.e., a novel environment) prior to exposure to a more severe stressor (i.e., chronic social stress) suffered larger impairments in memory. Additionally, other investigators have demonstrated the interaction

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Abbreviations: AcN, arcuate nucleus; AMYG, amygdala; AS, appetite suppression; BB, beam-break sensor; CA3, cornu ammonis 3; CORT, corticosterone; DA, dorsal area; ENK, enkephalin; EPM, elevated plus maze; HPA, hypothalamic–pituitary–adrenal; HPC, hippocampus; HT, hypothalamus; KRP, Krebs–Ringer phosphate; NPY, neuropeptide Y; PBS, phosphate-buffered saline; PTSD, post-traumatic stress disorder; RAM, radial arm maze; RBWG, reduced body weight gain; ROI, regions of interest; SSC, saline sodium citrate.

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http://dx.doi.org/10.1016/j.neuroscience.2013.04.052

between anxiety, stress, and memory (Sandi et al., 2008). Studies have shown that less anxious individuals learned better during a spatial memory task than those that were more anxious (Herrero et al., 2006).

While anxiety-related behaviors may predict individual differences in learning capabilities, it remains unclear, however, if pre-morbid anxiety can predict the degree of spatial memory that is retained after exposure to chronic severe stress, as well as other negative outcomes of stress. While identifying predisposed differences in particular behaviors that can serve as early indicators of susceptibility to stress has significant implications for refining clinical diagnosis of PTSD and establishing at-risk populations, understanding the neural mechanisms that play critical roles in governing stress resiliency and how they can be modulated is critical for developing treatments for such stress-related disorders (for review, see Southwick et al., 2005).

Neuropeptide Y (NPY) and enkephalin (ENK) are two neurochemical messengers of interest in the present study that have been implicated in playing critical roles in stress, memory, motivation, and anxiety across brain regions including the hypothalamus (HT), hippocampus (HPC), striatum, and amygdala (AMYG) (for review, see Charney, 2004). NPY has traditionally been studied for its metabolic role in modulating appetite and feeding behavior (Levine and Morley, 1984); however, it has more recently been studied for its interaction with hypothalamic–pituitary–adrenal (HPA)-axis activity, anxiety, and stress resiliency (Sainsbury et al., 1997; Sajdyk et al., 2008; Thorsell, 2010), in that NPY fosters anxiolytic and stress-resilient effects on behavior. Endogenous opioids including ENK have been studied for their roles in modulating aspects of motivation, reward, and memory in response to stress (Amir et al., 1980; Lucas et al., 2004, 2007), particularly in driving dopaminergic activity during reward–memory formation, but with blunted and decreased tone in the presence of chronic stress. How NPY and ENK are regulated in response to chronic stress and how these peptides might interact with predisposed differences in behavior in modulating individual differences in stress-induced outcomes across multiple physiological systems is still unclear. Revealing dysfunctions in the regulation of these neuropeptides in specific brain regions may provide insight into what aspects of stress resiliency are being governed by such circuits and if these peptides could serve as novel targets for pharmacological interventions.

The goal of the present study was threefold: our primary objective was to identify pre-morbid characteristics such as anxiety, locomotor reactivity, and learning traits that could be used to predict individual differences in stress-induced cognitive dysfunction; our secondary objective was to explore if stress resiliency spans across multiple systems to a similar degree within an individual, that is, whether stress resiliency within one particular domain (e.g., cognition) necessarily entails stress resiliency across other domains (e.g., metabolism, immune system); and, our third objective was to elucidate the regulatory roles of NPY and ENK neuropeptides in modulating stress resiliency. If a hallmark of PTSD in stress susceptible individuals includes prolonged affective dysfunction post-stress (e.g., anxiety-related dysfunction), we hypothesized then that perhaps increased pre-morbid sensitivity to stress (e.g., high anxiety and high locomotor reactivity) could serve as early indicators of susceptibility to stress. Given the widespread effects of stress on the brain and behavior and the heterogeneity of stress responses across physiological systems within an individual, we hypothesize that it is unlikely for an individual, stress-resilient within one domain, to maintain stress resiliency globally. Lastly, we suspected that elevated expression of NPY and ENK, based on their anxiolytic and motivation-promoting functions, would foster increased stress resiliency, perhaps only cognitively, and that deficiencies in such pathways may be indicative of increased susceptibility to stress and risk factors for stress-induced disease. In investigating the neural correlates of domain-specific stress resiliency, more may be revealed about the distinct roles such neural circuits play in managing various aspects of stress.

EXPERIMENTAL PROCEDURES

Behavioral analyses

Adult, male, Sprague–Dawley rats obtained from Charles River (Portage, MI, USA; $n = 52$) were bred in our animal care facilities and group-housed (three to six cohabitants) in clear, plastic cages ($47 \times 25.5 \times 21.5$ cm) with bedding until reaching approximately 2 months of age, ~200 g body weight. One week prior to the start of this experiment, animals were then individually housed. This study was divided into five phases structured around motivating animals to perform a spatial memory task, that is, to learn to locate a sugar reward using only spatial cues (Fig. 1). All phases of the experiment were run at 7:00 pm during these nocturnal animals' regular waking cycle. Animals were kept on a regular 12/12-h light/dark cycle (lights off at 6:00 pm, on at 6:00 am) with access to food (regular chow mix: pellet-typed LabDiet 5001 Rodent Diet [Southern Agriculture, Tulsa, OK, USA]) and water *ad libitum* until being placed on a food-restricted diet. Rooms were maintained at 22 °C, humidity 30–60%. Protocols in this experiment were approved by Loyola University Chicago's Institutional Animal Care and Use Committee (IACUC).

Phase A: restricted diet. Animals were placed on a restricted diet (15 g/d of regular chow mix) for 5 d until they reached approximately 85% body mass in order to make them appetitively motivated to seek a sugar reward during memory training. The restricted diet continued throughout the duration of memory training, but was slightly alleviated after initial weight loss (increased to 18.5 g/d) to ensure proper nutrition and growth during training. Animals always consumed their entire restricted-diet ration daily throughout the experiment. To acclimate subjects to the memory task's rewards, sugar treats (Kellogg's Froot Loops® cereal, 1