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Genetic vulnerability, timing of short-term stress and mood regulation: A rodent diffusion tensor imaging study

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Abstract
Early stressful life events predict depression and anxiety in carriers of specific polymorphisms and alter brain responses but brain structural phenotypes are largely unknown. We studied the interaction between short-term stress during specific time-windows and emotion-regulation using a genetic animal model of depression, the Wistar-Kyoto (WKY) rat. Brain structural alterations were analyzed using Diffusion Tensor Imaging (DTI). WKY (n=49) and Wistar (n=55) rats were divided into experimental groups: Early stress (ES): From postnatal day (PND) 27 rats were exposed to three consecutive days of stressors; Late stress (LS): From PND 44 rats were exposed to the same protocol; Control: No stressors. From PND 50, all animals were behaviorally tested for levels of anxiety and despair-like behaviors and then scanned. Gene × Environment × Timing ($G \times E \times T$) interactions (p=0.00022 after Hochberg correction) were found in ventral orbital cortex, cingulate cortex, external capsule, amygdala and dentate gyrus and in the emotion regulation measures. WKY showed longer immobility in forced swim test, but no effect of ES was detected. ES increased open-field anxiety-like behaviors in Wistar rats but not in WKY, possibly indicating a ceiling effect in WKY. Stress in pre-pubertal or adolescent phases in development may influence structural integrity of specific brain regions and emotion regulation behaviors depending on genetic vulnerability, consistent with a $G \times E \times T$ interaction in mood dysregulation.

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1. Introduction

Vulnerability to depression and anxiety is partly genetic and partly non-genetic due to familial and environmental risk factors (Kendler and Prescott, 1999; Kessler, 1997; Lupien et al., 2009; Nestler et al., 2002; Pine et al., 2002; Zalsman et al., 2006b). Stress-related neuroplastic changes in the brain of depressed patients have been reported (Nestler et al., 2002). Mood disorders result from genes that increase one's predisposition, gene–gene interactions, environmental risk factors (including early and current stressors) and gene-environment interactions (Costello et al., 2002; Mill and Petronis, 2007). Early stress (ES) produces alterations in brain structure and function that mediate the environment's effect on mood regulation and response to stress in adulthood. Brain vulnerability to ES is thought to decline with age of exposure, but little attention has been devoted to the relationship between age of ES and its impact on mood regulation and brain integrity (Baker et al., 2013). Mood dysregulation is a trait considered to be an early manifestation of mood disorders (Deveney et al., 2013; Posner et al., 2013; Ryan, 2013) and DSM 5 (APA, 2013) recently proposed a specific diagnosis, Disruptive Mood Dysregulation Disorder (DMDD), that may lead to a mood disorder later in life (Leibenluft, 2014).

The most studied candidate gene polymorphism in depression as an example of gene × environment interaction is the *5HTTLPR* in the promotor of the serotonin transporter gene. Children, adolescents and young adult carriers of the short (S)-allele are reported to be vulnerable to major depression when exposed to adverse events during childhood (Caspi et al., 2003; Eley et al., 2004; Kaufman et al., 2004; Kendler et al., 2005; Zalsman et al., 2006a). We replicated these findings (Zalsman et al., 2006a) in terms of severity of depression. Karg et al. (2011) in a meta-analysis of 54 studies confirmed that 5-HTTLPR polymorphism moderates the relationship between childhood stress and adult depression ($p=0.00002$). When stratifying the analysis by the type of stressor studied, they found strong evidence for an association between the S allele and increased stress sensitivity to childhood maltreatment ($p=0.00007$), but it was early but not late stress that was pathogenic.

During development there are sensitive periods in which the brain is more vulnerable to environmental influences (Knudsen, 2004). Giedd et al. (1999) used magnetic resonance imaging (MRI) in humans and showed a linear change in white matter across ages of 4 to 22, and nonlinear changes in cortical gray matter, with a preadolescence increase followed by a post adolescence decrease. Those developmental brain changes are evident not only in humans, but across species ranging from rodents to nonhuman primates (Spear, 2009) (for review see Buwalda et al., 2011). Stress during sensitive early childhood and adolescent periods affects normal brain development (Jankord et al., 2011; Lupien et al., 2009; Spear, 2009; Tottenham and Sheridan, 2009). Early-life stress with increased reactivity to stress may create cognitive and mood regulation deficits, with altered stress responses, in adulthood (Lupien et al., 2009).

Timing of childhood stress may be a crucial factor in modulating the gene × environment interaction. The present study hypothesizes (following Zalsman, 2010), that a study of gene × environment interaction must consider the timing of childhood stressors. We hypothesized that only when an individual with a specific genotype is exposed to a specific environmental risk, during a critical period of brain development, will depressive and anxiety symptoms emerge in adulthood.

In order to test this hypothesis we used Diffusion Tensor Imaging (DTI) analysis, which provides specific information on the architecture\microstructure of the tissue, based on measuring water molecules' motion or diffusion along multiple directions. We extracted two parameters: apparent diffusion coefficient (ADC), which measures the mean diffusivity, and fractional anisotropy (FA), which measures the directional preference of diffusion. Higher ADC levels refer to greater free diffusion, suggesting sparse cells or axons; higher FA levels suggest that the tissue is more organized (Assaf, 2008). DTI studies of mood disorders report lower anisotropy in the frontal and temporal lobes (Sexton et al., 2009).

Homologous brain structures are implicated in mood regulation for both humans and animals (Berridge and Waterhouse, 2003), allowing animal models to be used in depression research (Nestler and Hyman, 2010; Willner and Mitchell, 2002). The Wistar Kyoto (WKY) rat, a breed from the Wistar line, is stress-hyper-reactive, and is considered a "genetic animal model of depression", with anxiety-like behaviors (Malkesman and Weller, 2009; Pare, 1989a, 1989b, 1994a, 1994b). WKY rats demonstrate "behavioral despair" and "anhedonia" on several behavioral tests (Pare, 1989a, 1989b, 1994a, 1994b), two central criteria for depression diagnosis (Malkesman et al., 2006; Malkesman and Weller, 2009), as well as depression-like physiological and behavioral symptoms such as reduced body weight, disturbed REM sleep, lower levels of social behavior and social play than controls (Malkesman et al., 2006; Pare, 2000), and abnormalities in some central (monoaminergic) and peripheral (HPA axis) neurochemical systems (Jiao et al., 2003; O'Mahony et al., 2011; Scholl et al., 2010). We studied the interaction between genotype and short-term stress during specific time-windows on emotion-regulation and activity measures, using WKY and Wistar (control strain) rats. We then analyzed the changes in brain structure following early and late stress using DTI.

2. Experimental procedures

Rats from the "normal" control strain Wistar, and from the derived "depressive/anxious-like" line, WKY, were purchased from Harlan Labs Inc. and bred at the university's specific pathogen free animal housing facility. The facility meets NIH regulations; animal care is in accordance with the guidelines of the Society for Neuroscience and the American Psychological Association. Experiments meet National and European Animal Care regulations and were approved by the University's Animal Care and Use Committee. Rats were housed in a temperature controlled vivarium at 20-23 °C, under 12-h light-dark cycle (lights on at 0700). Food and water were available ad libitum. Housing was in Techniplast 42.5 × 26.6 × 18.5 cm cages with a shelter made from PVC piping. Litter size was culled on postnatal day (PND) 1−2 to 10+2, with approximately balanced sex-distribution. Pups were weaned on PND 22. For this study only males were selected. After weaning, animals were marked on the base of the tail with a color code using non-toxic markers

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