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Inflammatory Factors Mediate Vulnerability to a Social Stress-Induced Depressive-like Phenotype in Passive Coping Rats

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ABSTRACT

BACKGROUND: Coping strategy impacts susceptibility to psychosocial stress. The locus coeruleus (LC) and dorsal raphe (DR) are monoamine nuclei implicated in stress-related disorders. Our goal was to identify genes in these nuclei that distinguish active and passive coping strategies in response to social stress.

METHODS: Rats were exposed to repeated resident-intruder stress and coping strategy determined. Gene and protein expression in the LC and DR were determined by polymerase chain reaction array and enzyme-linked immunosorbent assay and compared between active and passive stress-coping and unstressed rats. The effect of daily interleukin (IL)-1 receptor antagonist before stress on anhedonia was also determined.

RESULTS: Rats exhibited passive or active coping strategies based on a short latency (SL) or longer latency (LL) to assume a defeat posture, respectively. Stress differentially regulated 19 and 26 genes in the LC and DR of SL and LL rats, respectively, many of which encoded for inflammatory factors. Notably, *Il-1β* was increased in SL and decreased in LL rats in both the LC and DR. Protein changes were generally consistent with a proinflammatory response to stress in SL rats selectively. Stress produced anhedonia selectively in SL rats and this was prevented by IL-1 receptor antagonist, consistent with a role for IL-1β in stress vulnerability.

CONCLUSIONS: This study highlighted distinctions in gene expression related to coping strategy in response to social stress. Passive coping was associated with a bias toward proinflammatory processes, particularly IL-1β, whereas active coping and resistance to stress-related pathology was associated with suppression of inflammatory processes.

Keywords: Affective disorders, Coping, Inflammation, Interleukin 1β, Social defeat, Susceptibility

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Stressors of a social nature are a common form of stress for humans, including abuse and bullying (1). The inability to successfully adapt to stress produces pathological changes that can lead to psychological disorders such as depression and anxiety and comorbid medical disorders including diabetes, irritable bowel syndrome, and cardiovascular disease (2–7). Interestingly, striking individual differences arise in the pathogenic potential of a stressor, rending one more or less likely to develop stress-related pathologies. Evidence suggests that individual differences in stress-induced pathology are related to coping style. For example, a submissive personality characterized by passive coping has been associated with vulnerability to psychopathology (8,9), irritable bowel syndrome (10), and hypertension (11–13), while active coping has been related to increased resiliency (14). Therefore, identifying the biology underlying different coping mechanisms may reveal systems or substrates that determine resilience or vulnerability to stress-related pathologies.

Social stress has been modeled in rodents through the use of the resident-intruder paradigm (15). We previously reported that, like humans, robust individual differences in the coping response to social stress emerge in an outbred population of Sprague Dawley rats, resulting in two phenotypes (16,17). One phenotype exhibits passive coping behaviors characterized by the assumption of a supine defeat posture within a short latency (SL). The SL phenotype develops stress-induced behavioral, neuroendocrine, and cardiovascular changes similar to those occurring during depression (16,18). In contrast, the alternate phenotype adopts a proactive coping strategy resulting in increased defeat latencies (long latency [LL] rats) and more upright postures and displays a general resistance to many of the pathological consequences observed in the SL phenotype (16–18). Phenotypic differences in coping mechanisms appear to have a strong genetic link because artificial selection for either extreme results in distinct genotypes within only a few generations (19). Furthermore, it has long been recognized that genetic differences in humans may affect vulnerability to psychiatric disorders, in part by influencing coping behaviors (20). Our previous work has highlighted numerous peripheral and central social stress-induced adaptations that occur in only a subpopulation of the same rat strain, suggesting that genetic variability within the outbred

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population may be driving these differences (16,17). The stress-sensitive brain regions, the locus coeruleus (LC) and dorsal raphe nucleus (DR), are source nuclei of the major brain monoamines that play a role in the behavioral stress coping response (21–25). Importantly, these two monoaminergic systems are also implicated in the pathophysiology of depression and therefore represent candidate brain regions that link stress to psychopathology. The unique passive stress coping style of the Wistar Kyoto rat was previously shown to exhibit differential gene expression in the LC and DR (26). More recently, we reported opposing stress-induced neuroadaptations in the corticotropin-releasing factor system within the DR of SL versus LL rats (17). Therefore, the present study compared the effects of stress on gene expression within the LC and DR of SL, LL, and control rats. Notably, due to opposing adaptations observed in genes encoding for inflammatory factors within the brain, these studies further identified changes in circulating and brain levels of inflammatory proteins and tested a causal role for interleukin (IL)-1β in a pathological consequence of stress.

METHODS AND MATERIALS

Animals

Male Sprague Dawley rats (225–250 g; intruder or control rats) and Long-Evans retired breeders (650–850 g; residents) (Charles River, Wilmington, Massachusetts) were individually housed in standard cages with free access to food and water on a 12-hour light/dark cycle. The Institutional Animal Care and Use Committee at the Children's Hospital of Philadelphia and the University of South Carolina approved these studies and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Social Stress (Resident-Intruder) Model

The social defeat procedure used was a modified version of the resident-intruder paradigm originally developed by Miczek (15) and identical to that used in our previous studies (16–18). Briefly, rats were randomly assigned to the intruder or control group. Intruders were exposed to a novel Long-Evans retired breeder for 30 minutes on 5 consecutive days. All Long-Evans rats were prescreened for their level of aggression and were only used in this study if they attacked within 60 seconds. The control group was exposed to a novel cage for 30 minutes daily. Average defeat latencies (latency to exhibit a supine submissive posture) were recorded. Intruder and control rats used in the gene expression studies were euthanized 24 hours after the fifth control or defeat exposure. Separate cohorts of rats were euthanized either 1 hour or 24 hours after the final social stress or control exposure for protein analysis. For the IL-1 receptor antagonist (IL-1ra) treatment study, a separate cohort of rats was euthanized 11 days after the final social defeat or control exposure.

Tissue Collection

Brains were sliced coronally until caudal LC (Bregma −10.08) (27) and DR (Bregma −8.04). For polymerase chain reaction (PCR) analysis, LC sections were mounted onto RNAse-free slides (Fisher Scientific, Waltham, Massachusetts) and LC tissue was micro-chiseled using an Eppendorf MicroDissector (Eppendorf, Hauppauge, New York). DR tissue was collected using a 2 mm punch at the midline just below the aqueduct using a 1 mm wide trephine. LC and DR tissue was stored in RNALater (Qiagen, Valencia, California) at −80°C. For protein analysis, the LC and DR were microdissected using a 1 mm wide trephine at 1 mm or 2 mm depth, respectively, and stored at −80°C.

RNA Isolation and PCR Array

Total RNA was isolated using Qiagen RNeasy Micro kit as per the manufacturer's instructions. The quality and concentration of final RNA yield was determined by using a ND-1000 NanoDrop UV spectrophotometer (ThermoFisher, Wilmington, Delaware) and 2100 Bioanalyzer RIN (Agilent Technologies, Santa Clara, California). RNA was converted to complementary DNA using the RT^2 First Strand Kit (SABiosciences, Valencia, California). Samples were run on a quantitative RT^2 Profiler PCR array containing 88 genes involved in G-protein coupled receptor signaling (GPCR Signaling PathwayFinder; SABiosciences) and run on a real-time PCR machine (SDS-7500; Applied Biosystems, Foster City, California). Data were quantified using the comparative cycle threshold method and normalized to the housekeeping genes *Hprt1* and *Ldha*.

Ingenuity Pathway Analysis

Fold change data were analyzed using the Ingenuity Pathway Analysis software (Ingenuity Systems, Redwood City, California) to identify biological networks of genes that were altered greater than 1.5-fold in socially stressed rats compared with control rats. A Fisher's exact test (p value $< .05$) was calculated to validate the association of a biological disease.

Determination of Cytokine Levels

LC and DR tissue were homogenized in RIPA buffer (Sigma, St Louis, Missouri) with protease and phosphatase inhibitor (Pierce, Rockford, Illinois). Homogenized brain tissue and plasma were assayed for IL-1β (R&D Systems, Minneapolis, Minnesota), monocyte chemoattractant protein-1 (MCP-1) and IL-10 (Invitrogen, Camarillo, California), and IL-6 (Pierce Biotechnology) using an enzyme-linked immunosorbent assay according to each manufacturer's protocol. Each protein of interest was expressed as a ratio to total protein.

Intracerebroventricular IL-1 Receptor Antagonist Treatment

A permanent guide cannula (22 gauge; Plastics One, Roanoke, Virginia) was implanted into the right lateral ventricle (anterior-posterior, −.8 mm relative to bregma; medial-lateral, −1.5 mm; dorsal-ventral, −4.2 mm from the skull). Rats were allowed 10 days to recover, at which time the innate immune response to the cannula is absent or substantially diminished (28). Histological verification of cannula placement was verified by injecting 5 μL of 1% Chicago Sky Blue (Alfa Aesar, Ward Hill, Massachusetts).

Rats were microinjected with 2.5 μL (.25 μg/rat intracerebroventricular, infused over 3 minutes) of recombinant rat IL-1ra (R&D Systems) or vehicle (.1% bovine serum albumin in sterile