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Strain dependent effects of prenatal stress on gene expression in the rat hippocampus

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ABSTRACT

Multiple animal models have been developed to recapitulate phenotypes of the human disease, schizophrenia. A model that simulates many of the cognitive and sensory deficits of the disorder is the use of random variable prenatal stress (PS) in the rat. These deficits suggest a molecular origin in the hippocampus, a brain region that plays a role in the regulation of stress. To study both hippocampal gene expression changes in offspring of prenatally stressed dams and to address genetic variability, we used a random array of prenatal stressors in three different rat strains with diverse responses to stress: Fischer, Sprague–Dawley, and Lewis rats. Candidate genes involved in stress, schizophrenia, cognition, neurotrophic effects, and immunity were selected for assessment by real-time quantitative PCR under resting conditions and following a brief exposure to restraint stress. PS resulted in significant differences in gene expression in the offspring that were strain dependent. mRNA expression for the N-methyl-D-aspartate receptor subtype 2B (*Grin2b*) was increased, and tumor necrosis factor-alpha (*Tnfα*) transcript was decreased in PS Sprague–Dawley and Lewis rats, but not in the Fischer rats. Expression of brain-derived neurotrophic factor (*Bdnf*) mRNA in the hippocampus was increased after an acute stress in all controls of each strain, yet a decrease was seen after acute stress in the PS Sprague–Dawley and Lewis rats. Expression of the glucocorticoid receptor (*Nr3c1*) was decreased in the Fischer strain when compared to Lewis or Sprague–Dawley rats, though the Fischer rats had markedly higher α7 nicotinic receptor (*Chrna7*) expression. The expression differences seen in these animals may be important elements of the phenotypic differences seen due to PS and genetic background.

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1. Introduction

Schizophrenia is a multifactorial illness affecting more than three million people, with contributions from both genetic and environmental factors. The disease is highly heritable [1], although thought to be multigenic [2,3]. Significant evidence of linkage to schizophrenia has been found at more than 15 chromosomal loci [4,5]. Implicated genes include brain-derived neurotrophic factor [6,7], glutamate receptors [8], and nicotinic receptors [9,10], all of which may play a role in the cognitive deficits seen in schizophrenia [11–13]. The environment also plays an important role in this developmental disorder [14]. Environmental factors such as stress, particularly during gestation, have been linked to schizophrenia [15–18].

Inherited endophenotypic traits, such as sensory processing of auditory stimuli [19] and eye-tracking deficits [20], are common in schizophrenic patients. Various models have been created to simulate these endophenotypes in animals [21]. One such animal model, the random variable PS paradigm, was developed to study the effects of prenatal stress (PS) on cognitive and sensory behavior and on gene expression in the offspring [22]. Administered in the last week of rat fetal development, it produces a phenotype with multiple features of schizophrenia including cognitive impairment, aberrant neuronal architecture, social withdrawal, altered stress responses, deficits in gating sensory information, disrupted pre-pulse inhibition, and increased response to amphetamines [23–25]. A previous microarray study of gene expression changes in this model was done in prefrontal cortex of Sprague–Dawley rats, demonstrating mRNA transcript alterations due to PS [26]. We have extended these studies to the hippocampus, following administration of the same random stress paradigm, because numerous studies have demonstrated pathologies in the hippocampus of patients with schizophrenia. Relevant candidate genes in five groups were evaluated: stress genes, candidate schizophrenia genes, genes involved in cognitive processes such as learning and memory, neurotrophins, and genes regulated in immune system signaling. In addition, three different rat strains with differing levels of stress responsivity were used to

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determine whether the genetic background of the animals would affect the outcome of expression changes. The strains were chosen based on their differing hormonal responses to stress: 1) Fischer rats exhibit hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis, 2) Lewis rats are known to be hypo-responsive to stress, and 3) Sprague–Dawley rats have intermediate corticosterone responses [27–31].

Six random stressors were administered during the last trimester to timed-pregnant rats. Male PS offspring at 56 days were either exposed to an acute stressor or not stressed, then sacrificed for study. The hippocampus was removed and utilized for gene expression assays. This region has been implicated in the pathology of schizophrenia [32,33], and in the cognitive effects of stress [34–36]. Of the genes assayed, significant effects of PS were found for *Grin2b*, *Tnfα*, and *Bdnf*, though the changes were strain dependent. Expression of *Chrna7* and *Nr3c1* was significantly different across rat strains, with no effects seen from PS.

2. Materials

Timed-pregnant rats were purchased from a breeding facility (Charles River, Kingston, NY) and arrived on the second gestational day (E2). Animals were given *ad libitum* access to food and water while on a 12 hour light/dark cycle. After parturition, dams and offspring were left undisturbed in large static cages until weaning on postnatal days (P) 23–25. There are no effects on litter size or birth weight with this random stress gestational model [25]. Males were then removed and housed with a single male littermate until the acute stress procedure and sacrifice on P56. All animal procedures were conducted in accordance with NIH policies and were approved by the University of Maryland, Baltimore Animal Care and Use Committee.

3. Methods

3.1. Prenatal stress procedure

Dams were randomly chosen to be placed in either a control or PS group. All dams in the PS group were exposed to a random variable stress paradigm for one week, from E14 to E21. Mild psychological and physiological stressors were given two or three times a day. All stressed dams received the same stressors at the same time of day. The stressors utilized were: 1) home cage placement in a cold room (4 °C) for 6 h, 2) 15 min of non-escapable swim, 3) 1 h restraint in a Plexiglas restrainer (Harvard Bioscience, Boston, MA), 4) 12 h of overnight food deprivation, 5) light cycle reversal, or 6) 12 h of cage overcrowding.

3.2. Postnatal acute stress

Male pups were sacrificed for the collection of hippocampal tissue and trunk blood by decapitation on P56. Sacrifice of both control and PS groups was performed at one of two time points: 1) at baseline without acute stress (baseline), or 2) immediately after a 30 min restraint stress (acute). At sacrifice, blood was collected in tubes containing the anticoagulant EDTA and plasma was harvested following centrifugation. The plasma was stored frozen at −80 °C until the corticosterone assay was performed.

3.3. Corticosterone measurement

Plasma corticosterone (CORT) concentrations were determined by radioimmunoassay according to manufacturer's instructions (MP Biomedicals, Orangeburg, NY). The assay has a sensitivity of 3 ng/ml, and an intra- and inter-assay coefficient of variation of less than 10%.

3.4. Tissue preparation and RNA isolation

After rapid decapitation, hippocampi composed of both dorsal and ventral structures were extracted from the brains by freehand dissection and placed in 2 ml of RNAlater solution (Ambion, Carlsbad, CA) to stabilize RNA for gene expression quantification. Hippocampi were stored at −80 °C prior to their transfer into tubes containing homogenization beads and TRIzol® lysis buffer (Invitrogen, Carlsbad, CA). Homogenization was accomplished using a Mini Beadbeater-8® (Biospec, Bartlesville, OK). Following tissue lysis, RNA was purified using an RNeasy mini kit (Qiagen, Valencia, CA). RNA quality was verified using an Agilent bioanalyzer 2100 (Agilent, Santa Clara, CA).

3.5. Quantification of mRNA levels by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Gene specific primers were designed to cross intron–exon boundaries and obtained from Eurofins Operon (Huntsville, AL). Primers used for the study are listed in Supplemental Table 1.

First strand complementary DNA (cDNA) was generated by reverse transcription using random hexamers and Superscript III reverse transcriptase (Invitrogen). qRT-PCR was performed on a Bio-rad iCycler IQ® (Bio-rad, Hercules, CA) using SYBRgreen supermix (Bio-rad). Each sample was run in triplicate, followed by a heat dissociation step to identify nonspecific products. Triplicates of each sample were averaged for each gene of interest (GOI) and then normalized to the housekeeping gene *Polr2a* (Pol) [37] using mean normalized expression (MNE) and the actual efficiencies (eff) of each run: $(\text{eff}_{\text{pol}})^{\text{CT}}/(\text{eff}_{\text{GOI}})^{\text{CT}} = \text{MNE}$, as described [38].

3.6. Statistical analysis

For all analyses, measures were expressed as means of each group ± SEM and compared using a mixed model analysis of variance (ANOVA) with fixed effects of strain, condition, and time group, plus a random effect of the offspring's dam (SAS foundation software V9, Cary, NC). Each group consisted of five to six male rats with each rat being taken from a different litter. Statistical differences between experimental groups were considered significant when $p<0.05$.

4. Results

4.1. Corticosterone response

At baseline there were no significant differences in CORT, either between strains or in the PS animals (Fig. 1). Each strain displayed the typical response to an acute stress with elevated CORT following a 30 min restraint stress. PS did not affect the CORT response in either the Fischer or Sprague–Dawley rats. However, in Lewis control and PS

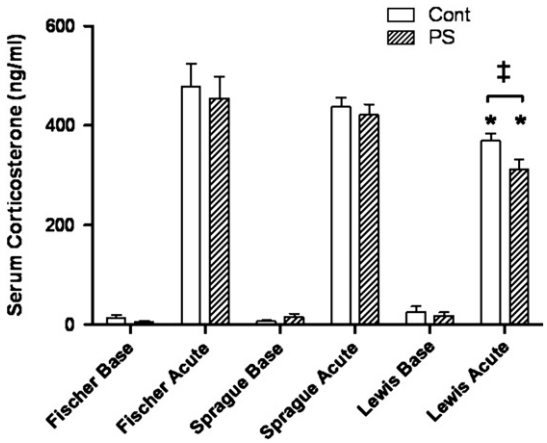

Fig. 1. Corticosterone levels before and immediately after an acute stress. Lewis control and PS animals have significantly lower serum CORT after an acute stress compared to Fischer or Sprague–Dawley rats (*, $p<0.05$). CORT levels were further reduced in Lewis PS rats, compared to the non-PS control (‡, $p<0.05$).