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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- α (*Tnfa*) 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 $\alpha 7$ 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.

Keywords

schizophrenia; rat model; prenatal stress; strain difference; *Grin2b*; *Nr3c1*; *Chrna7*; *Tnfa*; *Bdnf*

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

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[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 [11,12,13]. 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 [15,16,17,18].

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 [23,24,25]. 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 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 [27,28,29,30,31].

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 [34,35,36]. Of the genes assayed, significant effects of PS were found for *Grin2b*, *Tnfa*, 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 day (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 hours, 2) 15 minutes of non-escapable swim, 3) one hour restraint in a Plexiglas restrainer (Harvard Bioscience, Boston, MA), 4) twelve hours of overnight food deprivation, 5) light cycle reversal, or 6) twelve hours 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 mls 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 \pm 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 minute restraint stress. PS did not affect the CORT response in either the Fischer or Sprague-Dawley rats. However, in Lewis control and PS animals CORT levels were significantly lower than in the other two strains after the acute stress. Further, after the acute stress the Lewis PS animals had CORT levels that were significantly lower than the Lewis rat non-PS controls ($p < 0.05$).

4.2 Gene expression in the hippocampus

Candidate genes were selected to survey changes generated by the PS and/or the acute stressor, and to evaluate any rat strain differences. The gene groups, shown in Table 1, include genes known to play a role in stress or cognition, schizophrenia candidate genes, neurotrophins involved in development, and genes in the immune system implicated in stress and infection. Two genes were expressed at different levels across the strains and three were affected by PS in a strain-specific manner. Expression for all other genes listed was not significantly different in the PS animals nor between the rat strains.

4.2. *Grin2b*, *Tnfa*, and *Bdnf* hippocampal expression is altered by prenatal stress in a strain specific pattern

4.2.1. Hippocampal *Grin2b* expression levels are increased in prenatally stressed Sprague-Dawley and Lewis Rats—Expression of *Grin2b* mRNA was increased in offspring of PS Sprague-Dawley rat dams (Fig. 2; $p < 0.05$). The increase was found at both baseline and after acute stress, suggesting there was not a further increase due to the acute stress. In the Lewis rats, there was an increase in mRNA expression in the PS animals at baseline that did not reach significance. However, following exposure to an episode of restraint stress, an increase in hippocampal *Grin2b* mRNA levels was detected. Expression of *Grin2b* in the Fischer strain was not changed by PS or acute stress.

4.2.2. Expression of *Tnfa* is decreased by acute stress in prenatally stressed offspring—Of the genes in the immunity group, only the expression of hippocampal *Tnfa* mRNA was decreased following acute stress in PS Sprague-Dawley and Lewis rat strains (Fig. 3; $p < 0.05$). The decrease suggests that immune system responses in the hippocampus of PS animals are altered in these strains. There was no such change in expression of *Tnfa* mRNA in the PS Fischer rats.

4.2.3. Expression of *Bdnf* in the hippocampus is altered in the prenatally stressed Sprague-Dawley and Lewis strains, but not in the Fischer strain—Although the *Bdnf* gene is currently known to express multiple isoforms from its nine exons, there is one coding region identical to all transcripts which all translate to the same protein. We targeted our analysis to this coding region, and found that the expression of total *Bdnf*

mRNA was significantly increased by an acute stress in all three rat strain controls that were not exposed to PS (Fig. 4; $p < 0.05$), suggesting a hippocampal response to stressful events. However, the responses in the PS offspring were quite different and were strain dependent. *Bdnf* mRNA expression was significantly elevated at baseline in the PS Sprague-Dawley and Lewis rat strains (Fig. 4; $p < 0.05$), but was unchanged in the Fischer rats. There was no further change in *Bdnf* expression in the PS Sprague-Dawley or Fischer rats following acute restraint stress. However, *Bdnf* expression markedly declined in the PS Lewis rats (Fig. 4; $p < 0.05$) after acute stress, compared to respective baseline expression. The results suggest that *Bdnf* is differentially regulated in the three rat strains.

4.3. *Nr3c1* and *Chrna7* mRNA expression is significantly different across rat strains

Both *Nr3c1* and *Chrna7* mRNA transcripts are differentially expressed in the hippocampus of the three rat strains. *Nr3c1* mRNA was expressed at significantly lower levels in the Fischer rats at baseline, compared to both the Sprague-Dawley and Lewis rats (Fig. 5; $p < 0.05$). There was no effect of PS on gene expression in any of the rat strains. *Chrna7* was expressed at an approximately 50% higher level in the Fischer strain than in Sprague-Dawley or Lewis rats (Fig. 6; $p < 0.01$). Again, there was no effect of PS or the acute stress on mRNA expression levels.

5. Discussion

The use of six random stressors presented prenatally in the last week of fetal rat gestation results in altered phenotypes that are similar to those seen in schizophrenia [26,22]. We added genetic diversity to this paradigm by studying the effects of the stressors in three rat strains who differ in their stress response. Gestational stress exposure elicits enduring changes in the behavioral phenotype of the adult offspring. While repeated exposure to restraint stress results in a behavioral phenotype related to depression and anxiety, prenatal exposure to repeated mild, variable stressors produces a pattern of behavioral differences closer to those seen in schizophrenia [22]. Studies done by Kinnunen et al. [26] and Bogoch et al. [39] previously identified a number of gene expression differences associated with excitatory or inhibitory synaptic transmission in the frontal cortex and hippocampus. The present study extends these earlier findings by examining gene expression differences across three different animal strains. Since gene-environment interactions contribute prominently to neuropsychiatric illnesses, it is important to identify gene expression differences that can be attributed to genetic differences or environmental insults.

Nr3c1, also known as the glucocorticoid receptor, is important in regulating the negative feedback loop of the HPA-axis, and in its regulatory role of gene transcription. In our study, the Lewis rats had a smaller CORT response to an acute stress than the other strains. In addition, there was an up-regulation of *Nr3c1* in Lewis rats compared to Fischer. This suggests that having more receptors available might blunt the CORT response, possibly through increased negative feedback and tighter regulation, which is consistent with the known physiology of the HPA-axis [40]. The negative feedback signal that dampens the stress response may not be as robust in Fischer rats, allowing an enhanced CORT response to an acute stressor. Our results agree with three studies showing lower hippocampal *Nr3c1* mRNA expression in Fischer rats, compared to Lewis rats [29,41,42]. However, two studies show lower *Nr3c1* mRNA levels in Lewis rats [43,44], and another shows no difference between the two strains [45]. These differences may reflect genetic diversity due to vendor source or different techniques for analysis of mRNA expression. Our study used quantitative real-time PCR to study mRNA levels derived from both dorsal and ventral hippocampus, while others used *in situ* hybridization, northern blot, or cytosolic receptor binding assays.

Expression of *Grin2b* was increased in adult male offspring of PS Sprague-Dawley and Lewis rats. NMDA receptors are part of the ionotropic glutamate class of receptors; glutamate is the predominant excitatory neurotransmitter in the brain. The receptors are involved in long-term potentiation (LTP) and long-term depression (LTD), important measures of learning and memory [46]. NMDA receptors are heteromeric complexes with one *Grin1* subunit and at least one of four NR2 subunit types: *Grin2A*, *Grin2B*, *Grin2C*, or *Grin2D*. *Grin2B* is currently thought to be of particular importance for the induction of CA1 hippocampal LTD [47,48,49]. It is also known that stress can enable and facilitate LTD, and that blocking of *Grin2B* with specific antagonists can impair hippocampal CA1 LTD caused by acute stress [47]. In this model of PS, the expression of *Grin2B* was increased in both Sprague-Dawley and Lewis rats, but not in Fischer. Increased *Grin2B* expression would likely increase LTD and might be involved in the underlying mechanism for decreased cognitive performance in PS Sprague-Dawley rats [22].

Stress is a modifier of overall immune function [50,51] and cytokine transcription in the brain [52,53,54]. *Tnfa*, which can be down-regulated in the hippocampus by different stressors [55], is a pro-inflammatory cytokine normally involved in systemic inflammation and regulation of immune cells. Here, we see a rapid down-regulation of available transcript immediately following stress, demonstrating the tight regulation imposed due to the PS paradigm on the Lewis and Sprague-Dawley rats. Interestingly, *Tnfa* affects glutamate transmission, and plays a role in synaptic plasticity and LTP [56]. The cytokine can up-regulate AMPA receptors and simultaneously decrease synaptic inhibition through endocytosis of GABA receptors [57]. A decrease in *Tnfa*, as seen in our PS animals, may decrease the cognitive performance of these rats. *Tnfa* can also provide neuroprotective benefits in the brain. *Tnfa* stimulates activation of the transcription factor nuclear factor κ B (*Nfkb*), which may reduce cell death signals in neurons [58,59]. The reduction in *Tnfa* expression in the PS Sprague-Dawley and Lewis rats after an acute stress could negatively affect cell survival in these animals. We have previously found aberrant neuronal architecture in PS Sprague-Dawley rats [24]. Effects on brain development in the PS animals may be related to the inability of these animals to maintain *Tnfa* transcript levels after an acute stress.

Another developmental gene, *Bdnf*, was also affected by PS in the Sprague-Dawley and Lewis rats, but not in the Fischer. *Bdnf* is well known as an immediate early gene and its ability to respond quickly to stimuli. Interestingly, the 3'-UTR of *Bdnf* has the ability to either shorten or prolong the half-life of gene transcripts dependent on membrane depolarization [60]. *Bdnf* transcripts were increased in the non-PS controls of all three strains after an acute stress. However, the mRNA levels were significantly higher at baseline in Sprague-Dawley and Lewis rats in the PS offspring. Further, there was no up-regulation by acute stress in the PS animals in these strains as was seen in the controls. In the PS Lewis rats, acute stress resulted in a marked decrease in *Bdnf* expression, while there was no change in the PS Fischer rats. Although it is widely known that chronic stress or chronically elevated glucocorticoids can decrease hippocampal BDNF mRNA expression [61,62,63], the contrary is also true immediately after an acute stress, whereby there is a temporary increase in *BDNF* transcription as we see in our model [64,65]. *Bdnf* is initially expressed in a precursor form, pro*Bdnf*, which is processed to the mature neurotrophin, m*Bdnf* [66]. m*Bdnf* and pro*Bdnf* signal through two different receptors, the TrkB and p75 receptors, respectively. The activation of these receptors results in opposing cellular responses [67,68]. pro*BDNF* binding to p75 causes long-term depression, apoptosis, and synaptic retraction. However, m*BDNF* activation of the TrkB receptor results in the promotion of synaptic plasticity, cell survival, neuronal differentiation, and is sufficient for late-phase LTP while inhibiting LTD. Because these two differing signaling cascades come from the same *Bdnf* mRNA, examination of the *Bdnf* protein in PS animals would be of interest. We hypothesize

that mBdnf signaling would be decreased in PS animals, given that a decrease in these responses could contribute to the altered phenotype of the model.

The increased baseline transcript levels of *Bdnf* in the PS Lewis and Sprague-Dawley rats, as well as a failure of the PS strain to respond to an acute stressor with an increase in *Bdnf*, suggests that this rat strain may be less protected in inflammatory environments. This possibility is consistent with the differences in expression of *Chrna7* between the strains. *Chrna7* expression was 50% higher in the Fischer rats than in either the Sprague-Dawley or Lewis rats. The receptors expressed from this gene are protective for inflammation [69,70]. In addition, there is a relationship between stimulation of this receptor and *Tnfa* responses [71,72]. Thus, the low levels of *Chrna7* expression in Sprague-Dawley and Lewis rats may contribute to the differences in gene expression seen in these strains, compared to the Fischer rat.

Genetic diversity is a significant and fundamental obstacle in human studies, as are the effects of environmental factors such as stress on gene expression. Our results suggest that developmental pathways are affected by PS in the hippocampus with consequences that extend into adulthood, and that strain differences play an important role. The genes affected in the PS animals may be related to endophenotypic deficits seen in schizophrenia. Both *Chrna7* and *Bdnf* are replicated candidate genes in schizophrenia with decreased expression [73,74]. In addition, all the genes that were found to be altered in this study play a role in synaptic transmission pathways. The impact of PS on altered signaling in the hippocampus is, thus, an important consideration. Further investigation of the effects of PS on gene expression in the hippocampus and the interaction with genetic diversity will provide a better understanding of how brain development is affected by the environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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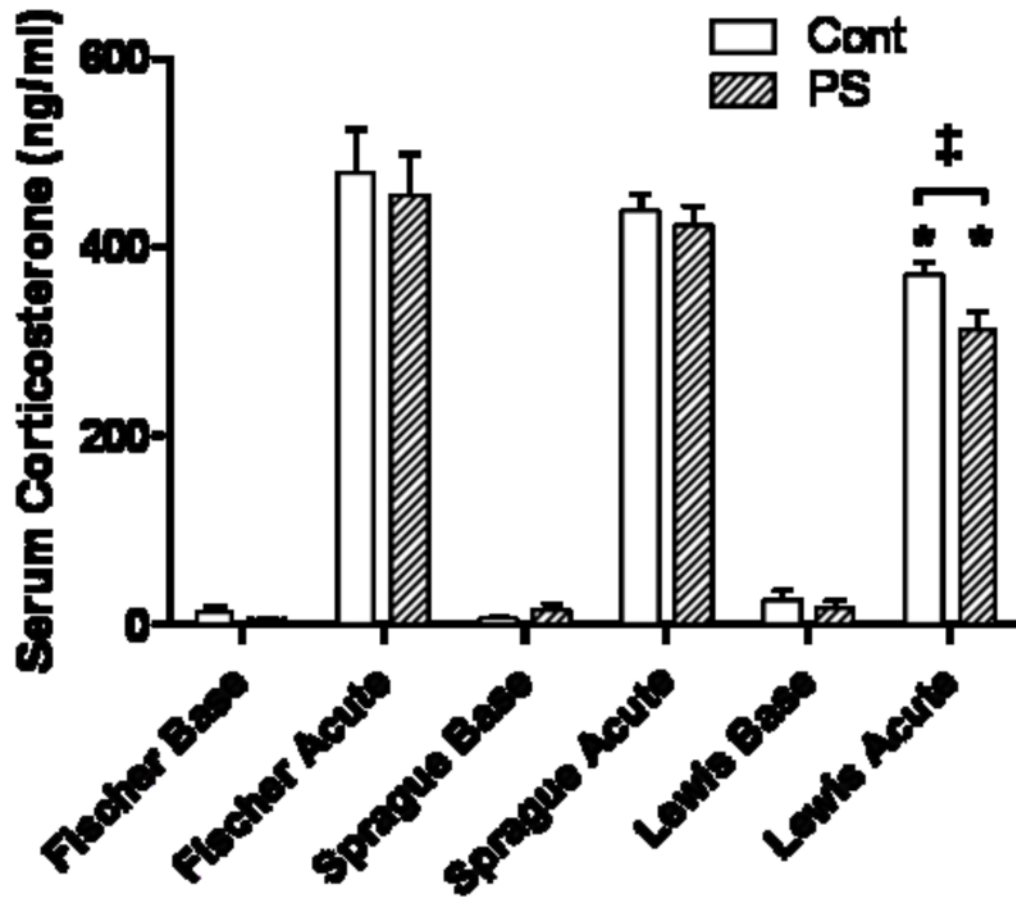


Figure 1. Corticosterone levels before and immediately after an acute stress
 Lewis control and PS animals have significantly lower serum CORT after an acute stress compared to Fischer or Sprague-Dawley rats (*, $p < 0.05$). CORT levels were further reduced in Lewis PS rats, compared to the non-PS control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

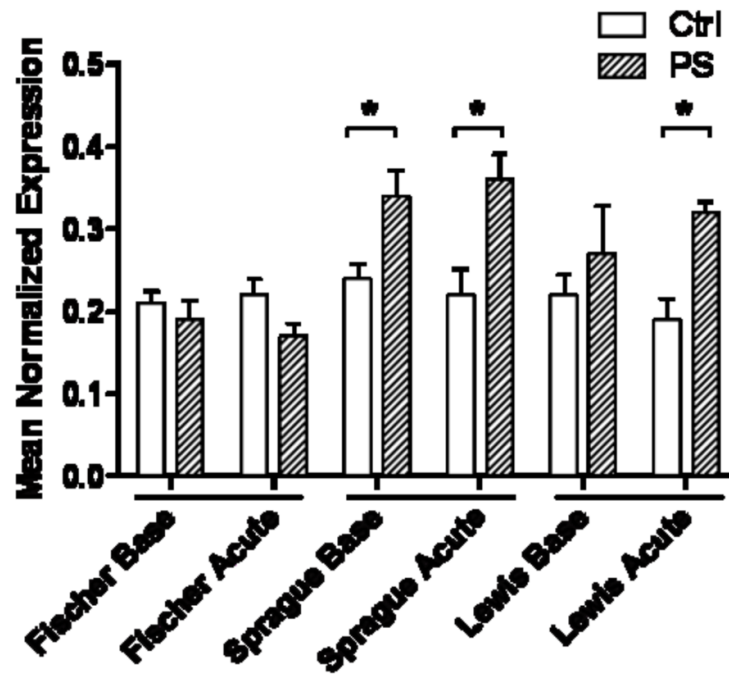


Figure 2. *Grin2b* mRNA levels in the hippocampus

Sprague-Dawley baseline and acute, plus Lewis acutely stressed, have up-regulated expression when compared to their respective controls (*, $p < 0.05$). Fischer rats were not changed.

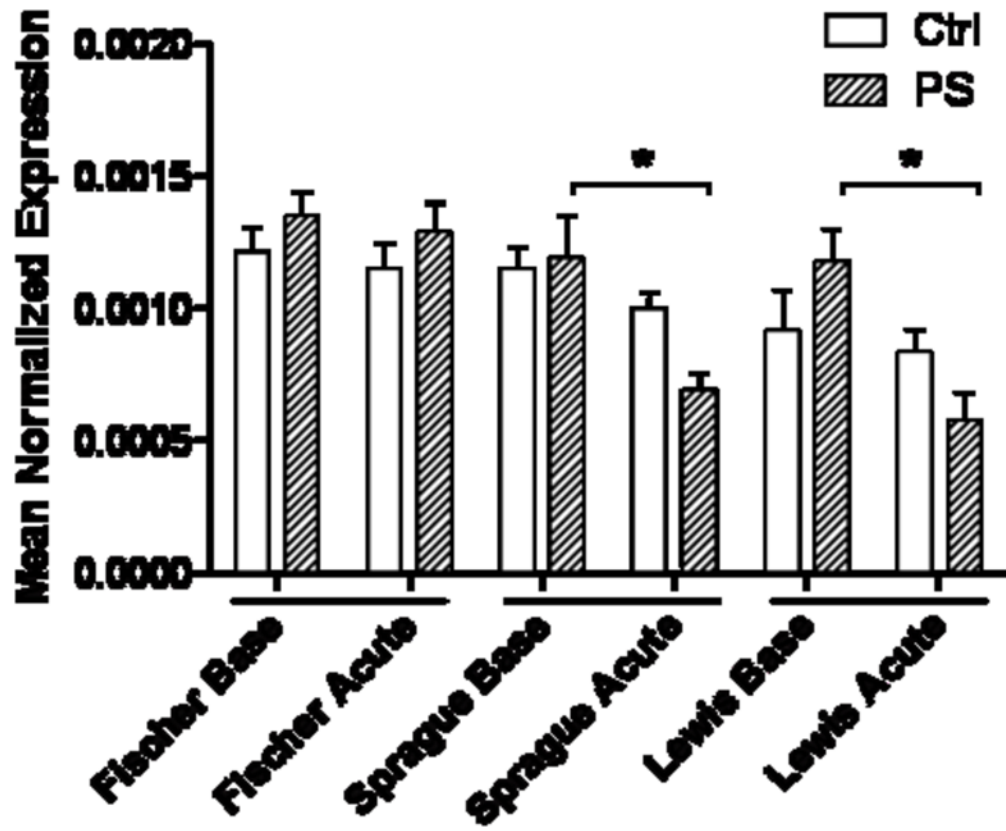


Figure 3. *Tnfa* mRNA levels in the hippocampus

Sprague-Dawley and Lewis rats exposed to PS show a significant decrease in expression of *Tnfa* after an acute stress that is not seen in the Fischer animals (*, $p < 0.05$).

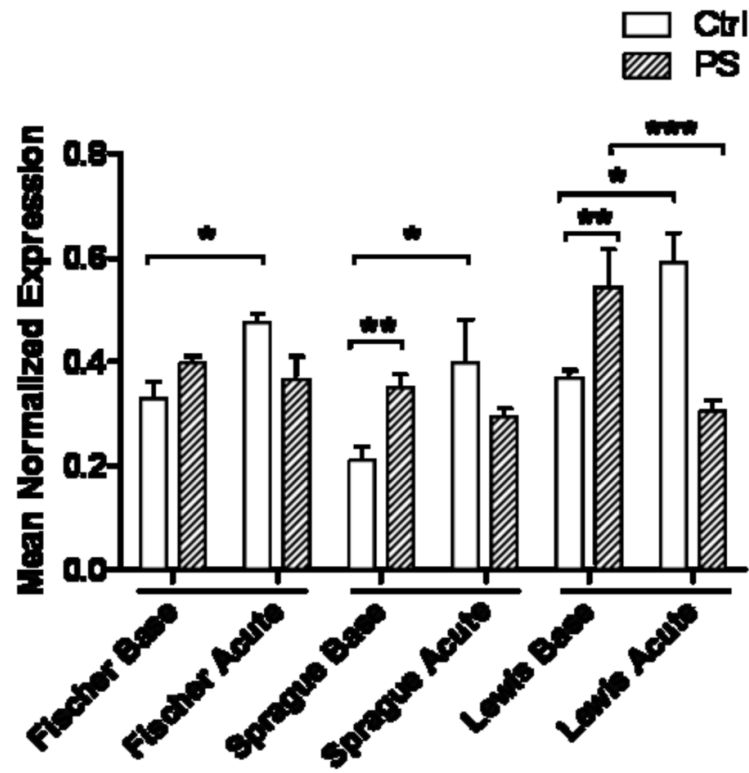


Figure 4. *Bdnf* mRNA expression in the hippocampus

Non-PS control animals of each strain significantly up-regulate *Bdnf* mRNA after an acute stress (*, $p < 0.05$). PS Sprague-Dawley and Lewis rats had higher baseline *Bdnf* expression (**, $p < 0.05$), which was unchanged after acute stress in the Sprague-Dawleys, but significantly decreased in the Lewis rats (***, $p < 0.05$). PS did not change expression in the Fischer strain.

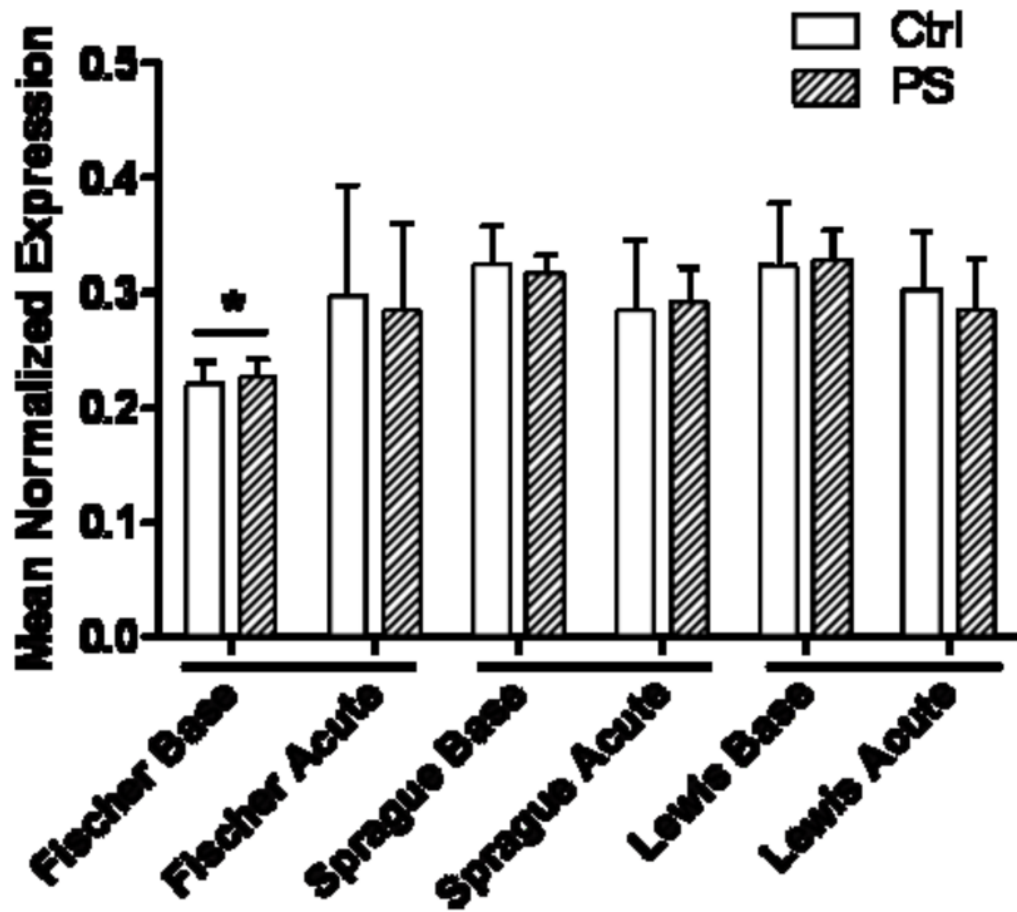


Figure 5. *Nr3c1* mRNA expression in the hippocampus

Fischer rats, regardless of PS or Ctrl group, have less *Nr3c1* transcript than either Sprague-Dawley or Lewis strains (*, $p < 0.05$).

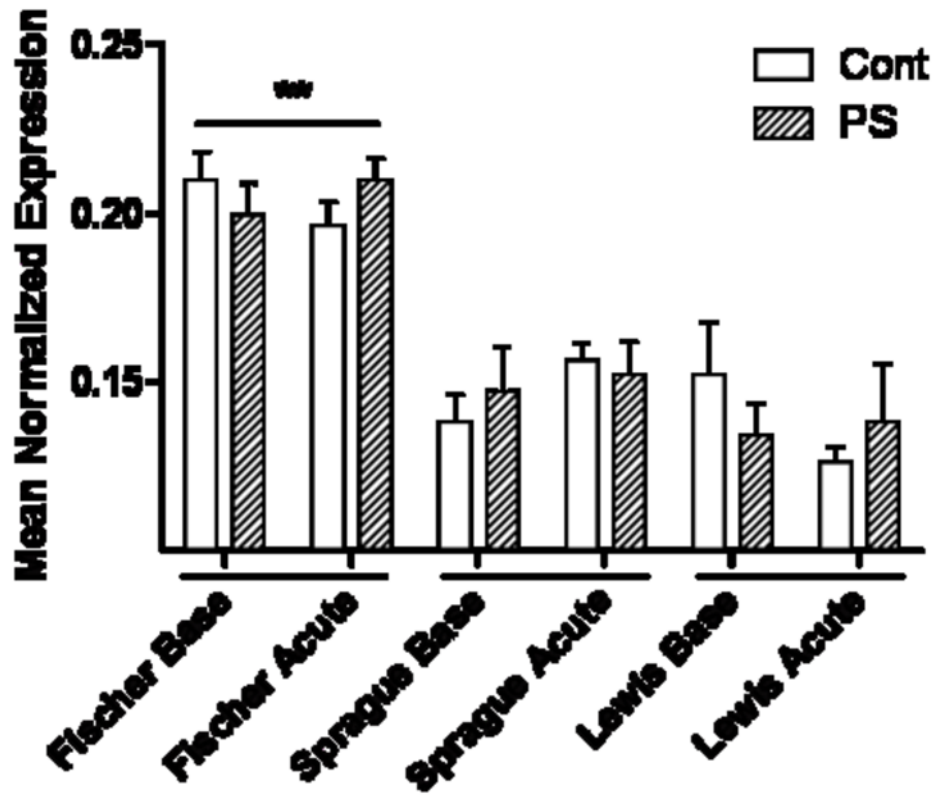


Figure 6. *Chrna7* mRNA expression in the hippocampus
Expression of *Chrna7* was significantly lower in Sprague-Dawley and Lewis than in Fischer rats (**, $p < 0.01$).

Table 1

Genes analyzed by qRT-PCR in the hippocampus of each animal group.

Stress	Schizophrenia	Cognition	Neurotrophins Immunity	
NR3C1*	CHRNA7*	GRIN1	BDNF**	TNFA**
NR3C2	RELN	GRIN2A	NGF	MR1
CRH	DTNBP1	GRIN2B**	NTF3	B2M
CRHR1	NRG1	GRIN2C	NTF5	IL1b
CRHBP	PPP3CA	GRIN2D	NTRK1	IL6
POMC	SYN2	GRINA	NTRK2	IL10
HSPB1	SYN3	GRIA1	NTRK3	
	COMT	GRIA2	MECP2	
	RGS4	GRIA3		
	DISC1	GRIA4		
		CAMK2A		
		CAMK2B		
		PRKACA		
		PRKACB		
		DLG4		
		LRRC7		
		NCAM1		

Significant differences were found for those in bold.

* different in rat strains;

** affected by PS