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Effects of restraint stress on the regulation of hippocampal glutamate receptor and inflammation genes in female C57BL/6 and BALB/c mice

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ABSTRACT

The two strains of inbred mice, BALB/c and C57BL/6, are widely used in pre-clinical psychiatry research due to their differences in stress susceptibility. Gene profiling studies in these strains have implicated the inflammation pathway as the main contributor to these differences. We focused our attention on female mice and tested their response to 5- or 10-day exposure to restraint stress. We examined the stress induced changes in the regulation of 11 inflammatory cytokine genes and 12 glutamate receptor genes in the hippocampus of female BALB/c and C57BL/6 mice using quantitative PCR. Elevated proinflammatory cytokine genes include Tumor Necrosis Factor alpha (TNFa), nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB), Interleukin 1 alpha (IL1a), Interleukin 1 receptor (IL1R), Interleukin 10 receptor alpha subunit (IL10Ra), Interleukin 10 receptor beta subunit (IL10Rb), and tumor necrosis factor (TNF) super family members. Our results show that BALB/c and C57BL/6 mice differ in the genes induced in response to stress exposure and the level of gene regulation change. Our results show that the gene regulation in female BALB/c and C57BL/6 mice differs between strains in the genes regulated and the magnitude of the changes.

1. Introduction

Rodent stress models are widely employed to investigate the role of stress exposure in psychiatric disorders. There is substantial evidence supporting the notion that stress is an important substrate for many of these illnesses (Yang et al., 2015). However, individuals' behavioral response to stress can differ greatly. Understanding the molecular mechanisms involved in differential stress response can shed light on how stress responses can result in either resiliency or susceptibility. Rodent strains that show differential responses to well understood stressors can be very useful in examining the genetic component of the disparity in stress susceptibility.

Two inbred mouse strains, BALB/c and C57BL/6, have been crucial in gaining an understanding of the behavioral and molecular responses to stress as they differ substantially in their response to stress. C57's are reported to be stress resilient in comparison to BALB/c, based in part to Balb/c exhibiting a heightened response to stress. For example, Balb/c show higher elevations in serum corticosterone levels (800–900 ng/ml) than C57's (450–500 ng/ml) upon exposure to restraint stress in both male and female mice (Flint and Tinkle, 2001). These strains have also been shown to differentially regulate hippocampal gene expression, which was associated with behavioral response in the chronic mild stress (CMS) paradigm where Balb/c's exhibited a significantly more pronounced depressive-like phenotype than C57's (Malki et al., 2015). Hippocampal gene profiling experiments have emphasized the importance of inflammation and immune pathways in stress susceptibility following CMS as well as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway in sensitization to stress in these two strains (Gray et al., 2013; Malki et al., 2015).

The link between stress-induced glucocorticoid elevation and glutamate release could be indicative of the genetic differences in stress susceptibility and potentially involve alterations in the regulation of glutamate receptors (Calabrese et al., 2012). Furthermore, the association between glutamate and inflammation has been well demonstrated (Pittenger et al., 2007). We previously showed that hippocampal gene expression, specifically inflammation and glutamate

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receptor genes, in male BALB/c and C57 mice, differed in the stress-induced genes that were dysregulated as well as the level of dysregulation (Sathyanesan et al., 2017).

Females have been shown to be at a greater risk of developing depressive disorders (Kornstein et al., 2000; Burt and Stein, 2002). Understanding the effects that cycling reproductive hormones have on the stress response could lead to a better understanding of this disparity. However, there is limited literature investigating the interactions of these systems and the female reproductive cycle, especially in relation to stress. We therefore performed hippocampal gene regulation studies pertaining to inflammatory and glutamate signaling cascades in naturally cycling, female Balb/c and C57 mice to gain insight on the interaction of the reproductive cycle with these gene targets and compare overall differences between males and females. We obtained quantitative PCR data on the genes of interest as well as estrous cycle data through lavage sampling. Methods for the experiments were identical to that of the ones described in Sathyanesan et al. (2017) on the basis that the ability to compare results from males and females are significant to understanding the underlying mechanisms and sex differences associated with MDD.

2. Methods and materials

2.1. Animals

Naturally cycling, female BALB/c and C57BL/6 mice aged two months (Envigo/Harlan, Research Models and Services, Indianapolis, IN, USA), 24 per strain, 17–21g body weight and group housed (4 per cage) in the same room in Techniplast blue line IVC cages with aspen chip bedding under standard conditions (20–23 °C, 40–70% humidity, lights on at 06:00 h and off at 20:00h) with unrestricted access to food and water. Mice were held in undisturbed quarantine for five days before experimentation. All animal use procedures were in strict accordance with the National Institutes of *Health Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committee at the University of South Dakota as well as in accordance to ARRIVE guidelines.

2.2. Estrous staging

Estrous samples were obtained by lavage on three consecutive days using nanopure water. Slides were dried, stained with cresyl violet for 15 s, rinsed to remove excess stain and left to dry. Stained slides were observed under a bright field microscope at 200x magnification and the estrous stage was identified according to previous publications (Caligioni, 2009; Byers et al., 2012).

Raw data from gene expression using quantitative PCR and grouped by estrous stage was statistically analyzed using the One Way ANOVA in Prism 7 (Prism 7, La Jolla, CA). Gene expression comparisons were considered statistically significant at $p < 0.05$. Significant effects were subjected to the Tukey Honest Significance Test.

2.3. Restraint stress

Mice were randomly assigned into three groups: 10 day restraint, 5 day restraint, and control (n = 8). Restraint stress using the Tailveiner Restrainer, length 10 cm, diameter 4 cm, slot ventilated (Braintree Scientific, Inc., Braintree, MA) began at approximately 14:00h each day for 2 h. The 5 day restraint group was exposed to stress on the 6th day of the experiment of the 10 day restraint group. The mice were returned to their home cage until the next session of restraint. Two hours after the final session, mice were killed by rapid decapitation per American Veterinary Medical Association guidelines (Leary et al., 2013). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques. The brains were hemisected, and the hippocampus was dissected and rapidly frozen on dry ice and stored at −80 °C until used in RNA isolation. The remaining intact hemisphere was also rapidly frozen until used in immunohistochemical analysis (Newton et al., 2003).

2.4. Quantitative PCR analysis

Quantitation of relative gene expression was performed as previously described (Sathyanesan et al., 2017) on whole hippocampus samples. Briefly, RNA was isolated using the RNAqueous kit (Ambion) and quantitated using the Nanodrop spectrophotometer (Thermo). Quality was confirmed using the Nanoassay on the Bioanalyzer (Agilent). Reverse transcribed cDNA was utilized for PCR amplification employing Sybr Green chemistry (Qiagen) and gene-specific primers in the Realplex Mastercycler realtime PCR machine (Eppendorf). Specificity of product was determined by melt curve analysis. Data were normalized using the housekeeping genes cyclophilin, β-actin and GAPDH.

2.5. Immunohistochemistry

Immunohistochemical studies were performed using cryocut coronal sections (n = 5 per group) (16 μm) as previously described (Sathyanesan et al., 2017). Briefly, sections were incubated with different primary antibody combinations in antibody solution, 2.5% bovine serum albumin (BSA) in phosphate buffered saline (PBS), at 4 °C overnight. Antibodies (TNFa and mGluR5, Abcam) were used as per manufacturer's instructions and specificity was tested using incubation in antibody solutions lacking primary antibody. Following primary antibody incubation, slides were washed in 1xPBS three times for 5 min each at room temperature. Slides were then incubated in appropriate fluorescent secondary antibody (1:500, Alexa- 594, Life Technologies) in 2.5% BSA in PBS for 2 h at room temperature. The slides were then rinsed in 1x PBS three times for 5 min each and coverslipped using VectaMount (Vector Labs). Three sections from each mouse were analyzed. Sections were viewed by an unbiased observer to evaluate differences and images were captured using a Nikon Eclipse Ni microscope equipped with a DS-Qi1 monochrome, cooled digital camera and NIS-AR 4.20 Elements imaging software. Objective magnifications that were used include 20× and 40x. Sections from stressed and control mice were captured using identical exposure settings. Regions of interest (ROI) in the images were quantitated using NIS-AR 4.20 software from Nikon. Image files were maintained in the native ND2 format.

2.6. Statistical analysis

Relative gene expression using quantitative PCR was calculated using the ΔΔCt method and the data were statistically analyzed using ANOVA in SigmaStat 4.0. Gene expression comparisons were considered statistically significant at $p < 0.05$. Significant effects were subjected to the Hohm-Sidak test for multiple comparisons. Results were replicated in an independent cohort. Data are presented as mean ± s.e.m.

3. Results

We studied stress induced hippocampal gene regulation in female BALB/c and C57BL/6 mice using quantitative PCR, focusing on glutamate and inflammation receptor genes. Our results indicate a limited correlation between estrous stage and gene regulation. Only 5 genes, GRIN1, GRIN2a, GRIN2b, GRIA1, GRIA2 exhibited regulation that correlated with estrous cycle. The relationship was observed only in C57BL/6 and restricted to the estrous phase. Distribution of individuals in each stage of the Estrous cycle are as follows: C57BL/6 10-day group (n = 8): Proestrus (n = 0), Estrus (n = 2), Metestrus (n = 2), Diestrus (n = 4), C57BL/6 5-day group (n = 8):Proestrus (n = 0), Estrus (n = 0), Metestrus (n = 2), Diestrus (n = 6), C57BL/6 Control group

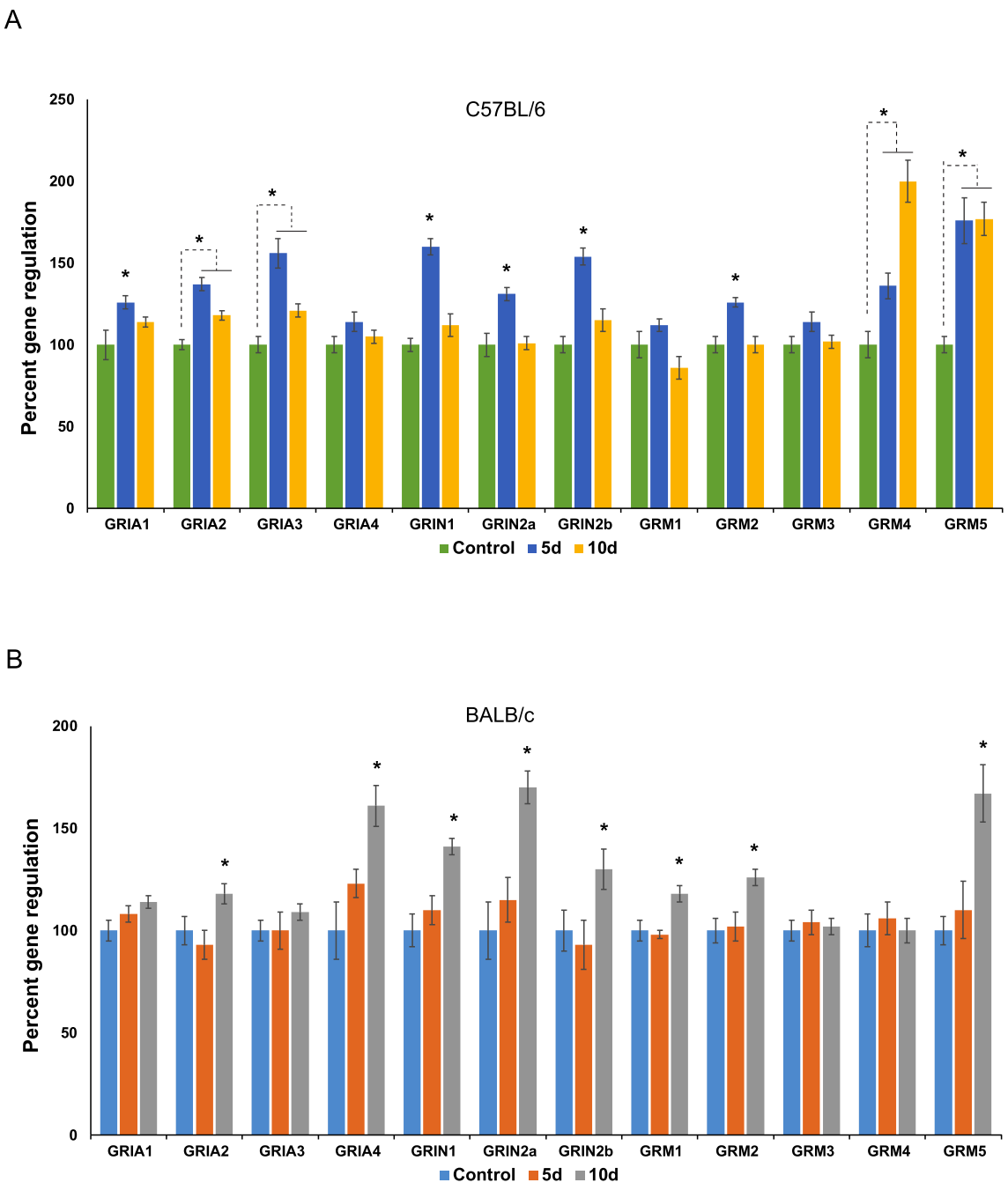

Fig. 1. Alterations in expression of hippocampal glutamate receptor genes after 5 and 10 days of restraint stress. (A) C57BL/6 mice. (B) BALB/c mice. Bars represent mean of N = 8. Error bars are ± SEM; *$p < 0.05$, ANOVA followed by Holm-Sidak test. GRIA1 (glutamate AMPA receptor), GRIA2 (AMPAR 2), GRIA3 (AMAPR 3), GRIA4 (AMPAR 4), GRIN1 (NMDAR 1), GRIN2a (NMDAR 2a), GRIN2b (NMDAR 2b), GRM1 (metabotropic glutamate receptor 1), GRM2 (metabotropic glutamate receptor 2), GRM3 (metabotropic glutamate receptor 3), GRM4 (metabotropic glutamate receptor 4) and GRM5 (metabotropic glutamate receptor 5).

(n = 8): Proestrus (n = 0), Estrus (n = 2), Metestrus (n = 1), Diestrus (n = 5); BALB/C 10-day group (n = 8): Proestrus (n = 4), Estrus (n = 4), Metestrus (n = 0), Diestrus (n = 0), BALB/C 5-day group (n = 7): Proestrus (n = 2), Estrus (n = 4), Metestrus (n = 1), Diestrus (n = 0), BALB/C Control group (n = 8): Proestrus (n = 1), Estrus (n = 5), Metestrus (n = 2), Diestrus (n = 0).

3.1. Glutamate receptor genes

After 10 days of restraint stress we found 4 upregulated glutamate receptor genes in C57BL/6 and 8 in BALB/c. GRIA2 (AMPA 2) and GRM5 were upregulated in both BALB/c and C57BL/6. GRM4, GRM5, and GRIN2a (mGluR 4 and 5, NMDAR 2a) showed the largest increase, all elevated above 150% compared to controls (Fig. 1A, B). We also tested the regulation of glutamate receptor genes after only 5 days of restraint stress. In C57BL/6 mice, all 4 genes that were upregulated in the 10 day group were elevated at 5 days (Fig. 1A). In addition, there were five more genes, GRIA1, GRIN1, GRIN2a, GRIN2b and GRM2, elevated after 5 days of stress exposure. In both 5 and 10 day groups, GRM4 and GRM5 were the most highly induced (Fig. 1A). In BALB/c mice, GRIA4 and GRIN2a from the 10 day group were also robustly elevated (Fig. 1B). In C57BL/6 there was an increase in gene expression in the 5 day group and then a return to control levels after 10 days in all genes except for GRM4 and GRM5, the former being greatly increased

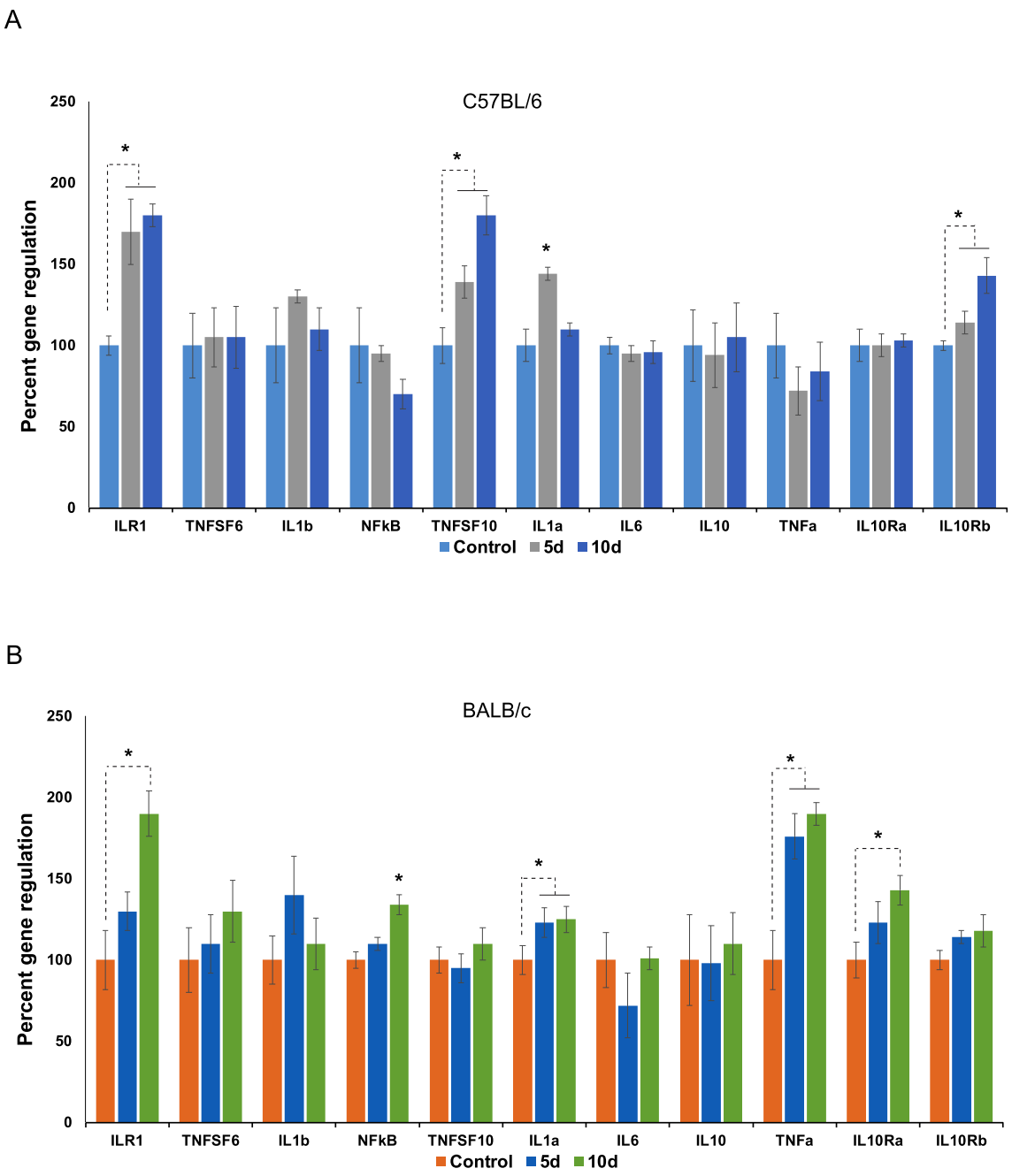

Fig. 2. Alterations in expression of hippocampal inflammation genes after 5 and 10 days of restraint stress. (A) C57BL/6 mice. (B) BALB/c mice. Bars represent mean of N = 8. Error bars are ± SEM; *p < 0.05, ANOVA followed by Holm-Sidak test. ILR1 (interleukin 1 receptor type 1), TNFSF 6, 10 (tumor necrosis factor super family 6, 10), IL1b (interleukin 1 beta), NFkB (nuclear factor kappa B subunit), IL1a (interleukin 1 alpha), IL6 (interleukin 6), IL10 (interleukin 10), TNFa (tumor necrosis factor alpha), IL10Ra (interleukin 10 receptor subunit alpha), IL10Rb (interleukin 10 receptor subunit beta).

after 10 days compared to 5 and the latter being similar after both 5 and 10 days (Fig. 1A). BALB/c on the other hand, showed levels close to that of the controls after 5 days but an increase in all significant genes at 10 days (Fig. 1B). Immunohistochemical analysis of GRM5 protein expression in C57's revealed that it was upregulated at 10 days in the stratum oriens and radiatum layers of the hippocampus (Fig. 3).

3.2. Inflammation genes

Ten days of restraint stress in C57BL/6 mice resulted in the upregulation of three genes associated with inflammation, ILR1, TNFSF10, and IL10RB. In BALB/c mice, five genes were upregulated, IL1R, NFKB, IL1a, TNFa, and IL10Ra. IL1R and TNFa showed the most elevated levels, 180% in comparison to controls.

We found that ILR1 and TNFSF10 were also robustly elevated after 5 days of restraint stress (Fig. 2). IL1a was induced only at the 5 day time point and returned to control levels at 10 days. In Balb/C mice, two of the five genes that were upregulated after 10 days were also upregulated after 5 days with none only upregulated after 5 days. Within the significant genes in BALB/c mice there appears to be a tendency for the genes to be elevated after 5 days but then elevated further after 10 days with the only exceptions being where the levels remained similar from the 5 day group to the 10 day group. Immunohistochemical analysis of TNFa expression in BALB/c mice showed that it was expressed at low

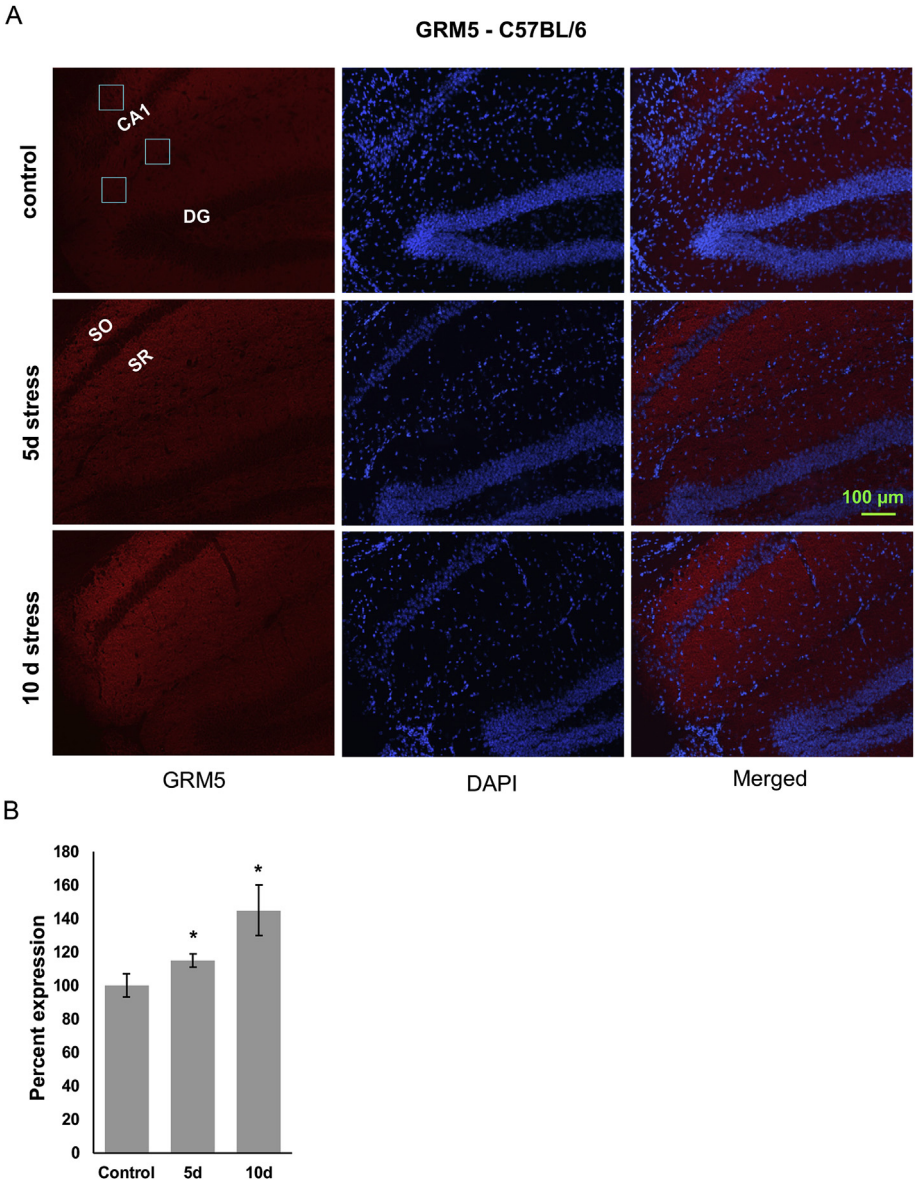

Fig. 3. Immunohistochemical analysis of GRM5 expression in the hippocampus. (A) The expression of metabotropic glutamate receptor 5 in C57BL/6 mice is shown in hippocampal sections from control (top panel), 5 days stress (middle panel) and 10 days stress exposure (bottom panel). Square boxes represent region of interest areas used for quantitation. CA1-cornu Ammonis, DG – dentate gyrus, SO – stratum oriens, SR – stratum radiatum. (B) Bar graph of relative quantitation ($N = 5$). Error bars are ± SEM; *$p < 0.05$.

levels in control animals, and highest expression was seen in the hippocampal dentate gyrus cell layer after 5 days of stress and returned to control levels at 10 days (Fig. 4).

4. Discussion

This study examined the effect of stress on the regulation of two specific classes of genes in two widely used female mouse strains. Restraint stress significantly increased the expression of several glutamate receptor genes in both strains. The effects were more pronounced in BALB/c than in C57BL/6 mice. BALB/c and C57BL/6 mice show distinct differences in behavioral tests and fear response under both native and stressed conditions (Malki et al., 2015). We found that some of the genes tested, such as IL-1a in C57 mice, were up-regulated after 5 days of stress exposure but then returned to baseline after 10 days. This phenomenon was prevalent in the glutamate receptor genes in C57 mice, but not in BALB/c. Our interpretation of this observation is that a return to baseline indicates an adaptive response to restraint stress. C57s are considered more stress resilient than BALB/c and adapt to the stressor after 5 days. In the (Nasca et al., 2017) study there was a robust downregulation of metabotropic glutamate receptor genes after a single acute exposure but recovered to baseline at 7 days in C57 males. Our current C57 results could be a habituation response as described by (Grissom and Bhatnagar, 2009) as the stressor is the same at 5 and 10 days. It will be interesting to test gene regulation with exposure to different stressors from days 5–10.

As stress models are heavily relied upon in preclinical studies of psychiatric disorders, it is useful to understand the molecular mechanisms underlying the differences in behavioral responses produced by stress in widely used mouse strains. It is also important to identify potential differences between males and females, especially due to the fact that females are at a greater risk for depressive disorders (Burt and Stein, 2002). Recently, there has been a surge in research studying these differences through a variety of stress paradigms (Bondar et al., 2018; Kuperman et al., 2016; Marrocco et al., 2017; Marchette et al., 2018). We believe that these comparisons can provide useful insight

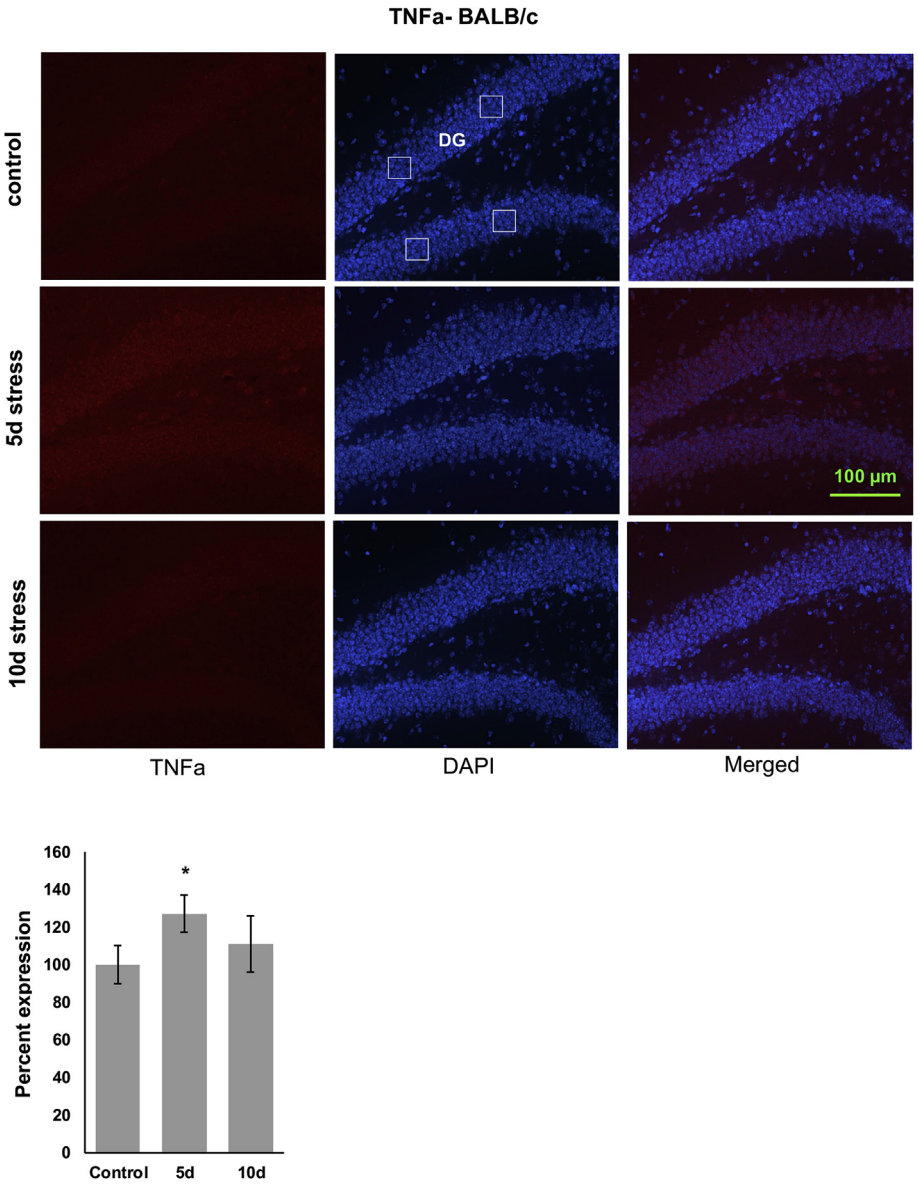

Fig. 4. Immunohistochemical analysis of TNFa expression in the hippocampus. (A) The expression of tumor necrosis factor alpha in BALB/c mice is shown in hippocampal sections from control (top panel), 5 days stress (middle panel) and 10 days stress exposure (bottom panel). Square boxes represent region of interest areas used for quantitation. DG – dentate gyrus. (B) Bar graph of relative quantitation (N = 5). Error bars are ± SEM; *p < 0.05.

and identify potential avenues for further research regarding sex-dependent differences in stress response and psychiatric diseases. While this study focused on the hippocampus, we plan to expand our areas of interest to include other related structures such as the prefrontal cortex and the nucleus accumbens.

4.1. Glutamate genes

Stress has been shown to dysregulate glutamatergic gene expression in the hippocampus, resulting in behavioral changes associated with psychiatric disorders (Nasca et al., 2015, 2017). Here, we show that stress differentially affected multiple classes of glutamate receptor genes in the two strains. Restraint stress differentially altered regulation of all three NMDA receptor subunit genes, GRIN1, GRIN2a, and GRIN2b in BALB/c and C57BL/6 mice. After five days of restraint stress these NMDA receptor genes were upregulated in C57s before returning to normal levels at ten days. This is contrary to what occurred in BALB/c where the genes remained at basal levels after five days of restraint and then increased with ten days of stress exposure. This could indicate that the time-dependent response of the stressor effects differs based on genetic background. The return to baseline in C57 s at 10d could indicate an adaptive response to stress, but is lacking in BALB/c. GRIN2a and GRIN2b were also elevated at 10d in male BALB/c but not in C57s (Sathyanesan et al., 2017). Interestingly, increased expression of GRIN2a and GRIN2b and several other glutamate receptor genes were reported in a recent large cohort postmortem gene expression study of the dorsolateral prefrontal cortex (DLPFC) in MDD patients, with particularly striking elevations in female MDD (Gray et al., 2015). However, it should be noted that there are also reports of decreased protein expression of GRIN2a and GRIN2b in the prefrontal cortex in MDD (Feyissa et al., 2009). The major impact of ketamine, an NMDA receptor antagonist, as an effective rapid acting antidepressant further suggests the importance of NMDA receptors in relation to MDD and its treatment (Duman, 2018). Rodent studies have reported that females are more sensitive to ketamine than males, and the heightened sensitivity is related to cyclic fluctuations in estradiol and progesterone levels (Saland et al., 2017).

The elevation in GRM2 (mGluR2) in both C57s (5d) and BALB/c

(10d) is noteworthy because currently mGlu 2/3 receptor antagonists are being studied as novel antidepressants (Dwyer et al., 2013). The differential regulation of GRM5 in C57s (10d), downregulated in males (Sathyanesan et al., 2017), but robust increase in females is quite intriguing as it mirrors the pattern of GRM5 regulation (down in males and up in females) in human MDD gene expression analysis (Gray et al., 2015). However, positron emission tomography (PET) imaging in MDD patients indicated lower levels of mGluR5 binding in multiple brain regions, including the cortex and hippocampus (Deschwanden et al., 2011). Somewhat paradoxically, another PET study reported that ketamine's antidepressant effects correlated with a decrease in mGluR5 binding, and was interpreted as due to receptor internalization (Esterlis et al., 2018). In our study, we see stress-induced increase in mGluR5 within the hippocampus, specifically in the CA1 stratum oriens and radiatum. This could suggest a potential neuroprotective reaction against glutamate toxicity via Long-term Depression (Snyder et al., 2001). The role of GRM5 in stress response, depression and antidepressant activity is likely to be complex, involving brain region and neuronal cell type specific effects. mGluR5 knockout mice exhibit a depressive-like phenotype after stress exposure while viral-mediated expression of mGluR5 in these mice promote resilience (Shin et al., 2015). Knockout of mGluR5 in glutamatergic neurons produces depression-like behaviors while knockout in GABAergic neurons elicits antidepressant-like effects (Lee et al., 2015). We also noted differences in mRNA and protein expression levels, particularly after 10d of restraint stress (C57s). We chose to focus on mGluR5 over the others owing to its relationship to MDD and active control of gene regulation.

5. Inflammation genes

The interplay between inflammation and depression has been well documented. Chronic inflammation being a signal for depression, involving the activation of pro-inflammatory cytokines (Fagundes et al., 2013; Derry et al., 2015; Kiecolt-Glaser et al., 2015). It is also known that stress exposure significantly influences the regulation of pro-inflammatory genes (Malki et al., 2015). Among the pro-inflammatory genes, the targets that have received considerable attention include Tumor Necrosis Factor alpha (TNFa), NFKB, and IL1B, all of which were regulated in our study. The effects were seen in both C57BL/6 and BALB/c mice but were more pronounced in BALB/c. This result supports our previous observation in male mice that simple restraint stress is sufficient to dysregulate hippocampal expression of inflammation genes (Sathyanesan et al., 2017). We also saw a hippocampal-layer specific localization of TNFa in the dentate gyrus of BALB/c mice. It is interesting to note that NF-kB was upregulated in BALB/c (10d group) but showed a trend towards downregulation in C57s (10d group).

IL10 (interleukin 10) is a known anti-inflammatory cytokine and is associated with the suppression of inflammatory cytokines. It is interesting to find that IL10 was not significantly upregulated in either strain, unlike the observation in male BALB/c mice where IL10 was sharply induced. For most of the genes that we examined expression appeared to be tightly regulated, with both five- and ten-day groups being close to controls. There was elevation of IL10 receptor subunits alpha and beta (IL10Ra and IL10Rb) in Balb/C and C57Bl6 respectively, both after ten days only. IL10Ra is the high affinity IL10 receptor while IL10Rb is a low affinity receptor that also participates in complex formation with other interleukin receptors such as IL20, IL22 and IL28 (Walter, 2014). In males, both IL10 receptors were elevated at 10d in BALB/c mice (Sathyanesan et al., 2017). Previous work in IL10 knockout mice has reported differential behavioral effects between male and female mice, with the females exhibiting a more depressive-like behavioral phenotype in the absence of IL10 (Mesquita et al., 2008).

The interleukin 1 receptor (IL1-R), which binds both IL1a and IL1b, two important inflammatory molecules associated with depression and other psychiatric disorders (Carvalho et al., 2014; Slavich and Irwin, 2014), was upregulated in both Balb/c and C57Bl6 mice at both time points. Signaling via this receptor has been demonstrated to produce downstream effects that results in increased NF-kB activity and increasing inflammatory responses (Verstrepen et al., 2008). This is intriguing because the rodent stress literature correlates chronic stress with an increase in NF-kB activity and expression. We observed a statistically significant increase in BALB/c after 10 days of restraint stress but a trend towards decrease in C57BL/6 mice. C57BL/6 males also showed NF-kB downregulation after 10d of stress exposure (Sathyanesan et al., 2017). Given the central role that NF-kB plays as a key transcription factor that mediates the inflammation pathway, the differential regulation of NF-kb could be involved in the stress susceptibility of BALB/c mice and the stress resiliency of C57Bl6's (Malki et al., 2015).

The association between depression and proinflammatory cytokines such as IL-6 and TNFa has attracted significant attention. A study involving over 1000 patients reported that levels of C-reactive protein (CRP), IL-6 and TNFa were higher in depressed men but not women, and highest levels of inflammation (levels of CRP and TNFa) were in men with late onset depression (Vogelzangs et al., 2012). Understanding the triggers and mechanisms involved in the dysregulation of cytokine levels in males and females will be important in devising novel therapeutic agents. The few clinical investigations that have examined the antidepressant effects of inflammatory cytokine blockade appear to be promising (Raison et al., 2013; Kappelmann et al., 2018). The elevation of TNFa that we observed after simple restraint stress could indicate that it is a primary response to stress exposure. The strain and sex dependent effects could provide a model for obtaining mechanistic insight into TNFa dysregulation and downstream signaling. The consequences of TNFa increase is likely to depend on the receptor through which it signals as its actions via TNFR1 vs TNFR2 are known to produce contrasting effects in response to elevated glutamate levels (Marchetti et al., 2004).

While mRNA levels remained high at 5 and 10d of stress, immunohistochemical detection of TNFa protein showed a return to baseline levels at 10d. Whether this is due to differential effects of stress on TNFa protein and mRNA levels is currently unclear and will be useful to examine in future studies. Overall, our analysis of stress-induced gene regulation in these widely studied mouse strains highlights female sex and strain specific effects. To further understand the implications of these changes stress-exposed female BALB/c and C57BL/6 mice can be tested in antidepressant-responsive behavioral assays with anti-inflammatory agents or rapid acting glutamate receptor drugs such as ketamine to determine antidepressant efficacy in a stress-altered background.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2019.100169.

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