

THE IMPACT OF EARLY LIFE STRESS ON INFLAMMATION  
AND EPIGENETICS IN ADULTHOOD

By

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AND EPIGENETICS IN ADULTHOOD

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## ABSTRACT

In 2020, nearly 8 in 10 adults said that the coronavirus pandemic was a significant stressor in their life, with nearly half of American adults reporting that their behavior has been negatively affected by stress. Stressors, much like the coronavirus, have been linked to altering acute and long-term inflammatory responses. One component of the stress response is inflammation. Inflammation acts as an adaptive part of the innate immune system that is activated in response to harmful stimuli. Though acute inflammation is critical in the process of fighting off disease and infection, prolonged inflammation has been shown to be correlated with neurodegenerative diseases such as Alzheimer's Disease. Exposure to stress at early periods of development, known as early life stress (ELS) has been shown to negatively impact the hypothalamic-pituitary-adrenal (HPA) axis, leading to disruption of the negative feedback loop. The goal of this study is to investigate the downstream effects of a combination early life stress model on targets and markers of the inflammatory pathway, including glucocorticoid receptor (GR) and DNMT1. Utilizing a restricted bedding stress paradigm, mice were exposed to stress prenatally and postnatally, with the prenatal stress induced via maternal stress. Preliminary results from this study indicate that the combination-stress (CS) condition resulted in an immunosuppressive effect given the downregulation of pro-inflammatory cytokines. However, quantification of data on GR suggests that exposure to stress early in life does not have a significant effect on levels of GR in the hippocampi of mice. Our study does provide support for increases in epigenetic modification in these CS mice with DNMT1 being present in higher levels in the CS mice compared to the no-stress (NS) control.

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## INTRODUCTION

Stress is generally defined as an intrinsic or extrinsic stimulus that disrupts the body's physiological homeostasis. Today, stress is a common part of everyday life for millions of individuals. According to the American Psychological Association in 2021, 84% of Americans report feeling emotions associated with stress in the last two weeks, with the most common emotions associated with the stress being anxiety (47%), sadness (44%) and anger (39%). While these data reflect stress associated with the coronavirus pandemic, stress has had a pronounced impact on society as overall stress levels have not changed significantly through the years. Despite stress having evolutionary benefits such as the acute survival capacities of the sympathetic nervous system response, stress can have detrimental outcomes depending on the acuity, duration, timing, and type of stressor (Lupien et al., 2009). Such outcomes include structural changes in brain morphology, changes in cognition and memory, and an increased propensity for neurodegenerative disorders (Yaribeygi et al., 2017).

A key component of the stress response pathway is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is an allostatic process aimed to maintain a narrow range of operation to ensure critical systems remained regulated. This process protects against environmental stressors and modulates biological processes such as blood pressure, heart rate, digestion, immune function, reproduction energy storage and growth. The HPA axis works through negative feedback regulation where neurons in the paraventricular nucleus (PVN) of the hypothalamus stimulate the release of corticotropin-releasing hormone (CRH) which targets the anterior pituitary gland. The anterior pituitary then releases adrenocorticotrophic hormone (ACTH), which acts on the adrenal gland to stimulate the production of glucocorticoid hormones. These glucocorticoids vary, with cortisol being the primary glucocorticoid in humans

and corticosterone being the primary glucocorticoid in rodents (Stephens et al., 2014). These glucocorticoids are then released into blood circulation and target tissues that regulate processes such as metabolism, immune function, skeletal growth, cardiovascular function, reproduction, and cognition (Ramamoorthy et al., 2016). To ensure the regulation of the HPA axis, glucocorticoids will act upstream on the PVN of the hypothalamus and the anterior pituitary to inhibit the release of CRH and ACTH which ultimately yields a decrease in glucocorticoids. This self-regulating mechanism prevents the exacerbation of the HPA axis and maintains stress related homeostasis.

Stress and immune function are tightly correlated. There is ample evidence to suggest that stress can activate the inflammatory response in the brain as well as in the periphery (Rohleder, 2014; Calcia et al., 2016). This response is an adaptive function of the innate immune system and is necessary to fight off harmful stimuli and pathogens. While inflammation is crucial for maintaining tissue homeostasis following bacterial invasion or tissue injury, prolonged activation of the inflammatory pathway provides a molecular basis for the pathology of many chronic diseases (Liu et al., 2017). The inflammatory response works through pro-inflammatory cytokines, which are small proteins secreted from macrophages and lymphocytes, and act in the up regulation of inflammatory reactions. Certain pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$  are crucial in this process and are noted to be involved in the process of pathological pain, cause immune cells to initiate phagocytosis, or secrete additional pro-inflammatory cytokines (Zhang et al., 2007; Chung et al., 2009; Netea et al., 2010).

Glucocorticoids themselves can have a profound impact on the inflammatory outcomes in response to stress. Glucocorticoids have been found to be involved in both immune activation and suppression, leading researchers to question how glucocorticoids can induce two opposing

mechanisms (Sorrells et al., 2007). Preliminary research suggests that basal and low levels of glucocorticoids and catecholamines are required for early increases in the immune response, while only at higher concentrations of glucocorticoids there are anti-inflammatory effects (Munck et al., 1984). One proposed mechanism for this dichotomous action suggests that glucocorticoids modulate the inflammatory response through the differential binding of both the mineralocorticoid and glucocorticoid receptors (GR) and subsequently induce differential regulation of NF- $\kappa$ B. Current research has shown there to be biphasic effects associated with glucocorticoid binding where there is a MR-dependent potentiation of pro-inflammatory cytokines and NF- $\kappa$ B activation at low to moderate concentrations and a GR-dependent suppression of pro-inflammatory cytokines and NF- $\kappa$ B activation at high concentrations (Chantong et al., 2012). Various other factors including timing and catecholamine release have been shown to impact the inflammatory process. When an acute stressor is presented before an inflammatory challenge, there is an increase in the inflammatory response. Conversely, an acute stressor presented after an inflammatory challenge dampens the inflammatory response. Catecholamines work to influence inflammation by serving as a primer for the stress-exacerbated immune response (Sorrells et al., 2007). Overall, stress and subsequent glucocorticoid release have a direct impact on inflammatory outcomes.

Stress at early stages of development have been shown to lead to alterations in the immune response later in life. Early life stress (ELS) can include both prenatal and postnatal stress and has been associated with an increased vulnerability to develop psychiatric disorders and impaired cognitive functioning in adulthood (Hoeijmakers et al., 2015). It is important to note that exposure to stress, particularly in the prenatal period, is greatly influenced by the timing and chronicity of the stressor. Exposure to stress during early periods of gestation have led to

behavioral impairments such as reduced locomotion and exploration whereas mice pups stressed during the final week of gestation showed more exploratory behavior than the non-stress controls (Meek et al. 2000). One study conducted in humans used 1872 participants from the Helenski Birth Cohort Study born in 1934-1944 to self-report anxiety symptoms at the age of 65-77. In this study, researchers examined the relationship of various forms of potentially stressful childhood experiences referred to as ELS – namely emotional and physical trauma, low socioeconomic status (SES), separation from parents, death of a family member, and parental divorce – with late adulthood anxiety symptoms. The study found that the accumulation of different early life stressors was associated with higher anxiety symptoms and the risk of clinically significant anxiety (Lahdepuro et al., 2019). Findings such as these substantiate the importance of understanding prenatal and postnatal stress as a long-term issue that proceeds into adulthood, establishing ELS as an important public health issue.

Previous rodent studies have shown that ELS can have a variety of effects on immune function. However, the timing of the stressor in relation to the developmental stage has significant implications on the pathology associated with the stressor. Many ELS events have immunological symptoms that do not present until later into adulthood, making it difficult to identify until well past the critical development window (Brunson et al., 2005). These studies have categorized the development of the immune system into different windows spanning through the 21-day gestation period, early postnatal suckling, weaning at postnatal day 21, and ending when mice reach 8 weeks of age (Dietert et al., 2000, Veru et al., 2014). In consideration of these critical periods of immune development, one study found that exposure to maternal separation from birth to postnatal day 15 led to an exacerbation of the cytokine response in infantile mice (Roque et al., 2016). While additional studies have examined the effects of

prenatal stress on the inflammatory response in adulthood, few have found alterations in inflammatory signaling that last until adulthood after maternal stress during gestation.

Research on the long-term implications of stress-induced inflammation on adult neuropathology is clinically relevant given the tight correlation between inflammation and the development of Alzheimer's pathology. In studies that look at this relationship, researchers have found that inflammation induced via daily injections of LPS, a trigger for the immune response, led to an increase in the accumulation of amyloid-beta (A $\beta$ ) plaques. These A $\beta$  plaques are the pathological marker for Alzheimer's disease. Even more, the production of A $\beta$  plaques induces more inflammation, creating a cyclical nature whereby inflammation induces A $\beta$  plaques which in turn creates more A $\beta$  plaques. Therefore, studies that look at the implications of ELS on inflammation later in life are clinically relevant as Alzheimer's disease affects over 6 million people in the United States alone.

Understanding the long-term clinical applications of ELS has been of great importance in recent years. Previous work in the lab explores how prenatal and postnatal stressors work to induce various inflammatory responses through a bedding restriction paradigm. To better elucidate the relationship between postnatal stress, prenatal and postnatal stress, and no stress, three different conditions were created. The first group of mice experienced stress during the entire prenatal period and with the early postnatal period. The second group of mice was exposed to stress during the early postnatal period, and the final group of mice was not exposed to additional stress at any point. For mice in the combination-stress condition, there was an immunosuppressive effect through downregulation of pro-inflammatory cytokines. These findings suggest potential alterations in the stress-mediated inflammatory response.

Knowing the preliminary data surrounding exposure to ELS and subsequent inflammatory dysregulation in adult mice, the potential role of epigenetic modification has become a target for further research. Epigenetics has been operationally defined as a process that results in changes in the chromosome without alterations in the DNA sequence that yields a stably heritable phenotype (Berger et al., 2009). These changes in the chromosome are noted for their ability to both increase and decrease gene expression, depending on the type of modification to the genome. There are three notable mechanisms by which epigenetics can lead to changes in gene expression and protein production. The first is DNA methylation, which is mediated by DNA methyltransferase enzymes at locations dense in cytosine known as CpG sites. Methylation at these sites is shown to decrease gene expression by reducing the binding of transcription factors or increasing the binding of methyl-CpG binding proteins. The second is histone acetylation. This modification occurs particularly in lysine residues of histone tails and can stimulate the binding of transcription factors and subsequent gene expression. Lastly, is miRNA. The formation of miRNA offers a mechanism to regulate gene expression at the mRNA level through the complementary binding and silencing of the gene at the target regions (Moosavi et al., 2016).

One potential hypothesis for the dysregulation of the inflammatory pathway in prenatally and postnatally stressed mice stems from foundational research on stress and epigenetics by Michael Meaney and colleagues. In their study, they looked at the implications of varied mother-infant interactions and how this led to differences in behavior and endocrine response to stress in adulthood. Their study analyzed the differences in licking-patterns between a mother and her offspring, with higher rates of licking being associated with higher levels of maternal care and investment. Conversely, lower licking rates were associated with lower levels of maternal care

and investment. Further investigation into pups of these mothers found differences in the DNA methylation patterns for offspring who had high versus low-licking mothers whereby high-licking resulted in less methylation and low-licking increased methylation. More specifically, these epigenetic modifications in pups exposed to low licking were found to have a decreased expression of GR induced through the modification of chromatin structure. This subsequently altered the stress response in these offspring through an increase in corticosterone and dysregulation of the HPA axis (Meaney et al., 2005). Another study looked more specifically at the methylation patterns of the brains of prenatally stressed mice and found that these mice had increased levels of DNMT1, which plays an important role in the maintenance of methylation (Benoit et al., 2015).

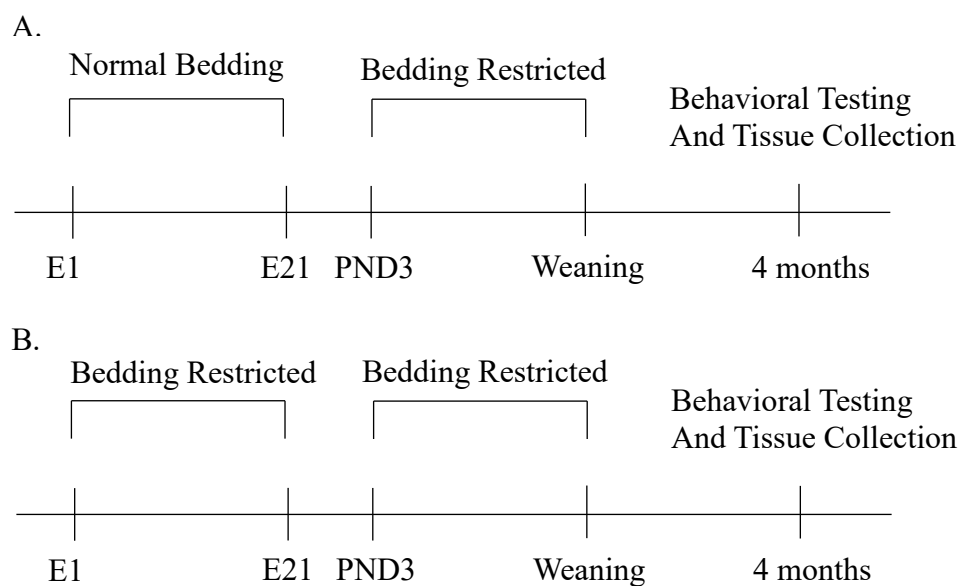
Given the previous findings that show alterations in pro-inflammatory cytokines in combination prenatal and postnatal stress mice compared to postnatally stressed and non-stressed mice, GR was hypothesized to be a potential target of methylation by a DNA methyltransferase. With GR's significant role in the regulation of both the HPA axis and the immune response, we believed this target to be a foundational step to better understanding the mechanism behind the changes seen between stress models. Ultimately, we hypothesized that stress induced via a combination of prenatal and postnatal bedding restriction will decrease the level of GR, which results in a blunting of the inflammatory response. This will result in higher levels of DNMT1 and lower levels of GR in the hippocampus compared to no-stress control animals.

## MATERIALS AND METHODS

### **Animals and Stress Paradigms**

This study utilized C57BL/6J male mice aged 4-6 months housed in the TCU vivarium in accordance with procedures for approved animal housing. Early life stress (ELS) was modeled through bedding restriction which was previously demonstrated to be an effective stressor model for early life (Johnson et al 2018). Mice undergoing bedding restriction were removed from their normal housing that included a 2x2 cotton nestlet, which mice normally use to construct nests to burrow in and placed in a cage without this nestlet. 2–3-month-old female mice were mated with 2–3-month-old sexually experienced males with females assigned to one of three conditions: early postnatal stress (PS), prenatal and early postnatal stress which was termed combination stress (CS), and no stress (NS). For mice in the postnatal stress condition, the dam and pups were moved to a cage without a bedding nestlet at postnatal day (PND) 3. They remained in this cage until weaning at PND21. For mice under the combination stress condition, dams were examined for the presence of a vaginal plug after the introduction of a mate. Presence of a vaginal plug confirmed successful mating and was considered embryonic day zero (E0). On E3, pregnant females were transferred to a cage without the bedding nestlet and remain in that cage until parturition. On PND0, the dam and pups were temporarily moved to a cage that included the nestlet until PND3 to protect against pup mortality. Mice were then transferred to cages without a nestlet from PND3 until weaning. Mice in the no stress condition underwent no housing changes. Pups were aged to adulthood (4-6 months) for behavior testing and biological analysis.

For this study, only the no stress and combined stress conditions were analyzed.



**Figure 1.** Bedding restriction timelines for the postnatal stress condition (A) and the combination stress condition (B). For the postnatal stress condition, mice were placed in housing with restricted bedding from postnatal day 3 to weaning (PND3-PND21). For the combination stress condition, mice were placed in housing with restricted bedding from embryonic day 1 to parturition (E1-E21) then returned to normal housing conditions until postnatal day 3 where they are placed back into housing with restricted bedding until weaning.

### Western Blot Analysis

Western blotting was used to assess changes in the protein concentrations of GR and DNMT1 in the hippocampal region. Protein concentration was measured using both a GR antibody and a DNMT1 antibody. GR is a 97kDa mediator of the immune response in the hippocampal region. DNMT1 is a 183 kDa DNA methyltransferase hypothesized to target GR in the hippocampus. First, lysates were diluted with sample buffer and boiled for five minutes at 100 °C and transferred to ice. Samples (0.5mg/1ml) were centrifuged and then loaded into 4–20% Mini-PROTEAN TGX polyacrylamide gels (BioRad, Hercules, CA). Samples from each stress condition were included in each blot. Gels were loaded into an electrode holder and placed

in the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS Page) apparatus containing SDS running buffer. Gel electrophoresis was run at 200 volts, 300 watts, and 3.0 amps for 55 minutes. Subsequently, the gel was transferred to a 0.45 $\mu$ m Hybond PVDF membranes (Genesee Scientific, San Diego, CA) and blocked with 5% BSA (albumin, bovine fraction) in Tris-buffered saline with Tween-20 (BSA; Research Products International, Mount Prospect, IL). Next, the membrane was treated with either glucocorticoid receptor primary antibody (Glucocorticoid Receptor Polyclonal Antibody; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) at a 1:750 dilution or DNMT1 primary antibody (DNMT1 Polyclonal Antibody; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) at a dilution of 1:750 in Tris-Buffered Saline and Tween Detergent (TBST) and incubated at 40 C overnight. Afterwards, the membrane was washed in TBST for one hour and treated with goat anti-mouse IgG, secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) at a dilution of 1:25,000 in TBST for two hours. Finally, the membrane was washed in TBST for one hour and treated with chemiluminescent solution (SuperSignal West Pico PLUS Chemiluminescent Substrate, ThermoScientific, Waltham, MA), and imaged (G:Box Chemi ST 4, Syngene, Frederick, MD). Alpha tubulin (at a dilution of 1:10,000 in TBST; Santa Cruz Biotechnology) was utilized as the internal reference protein, and densitometry was performed using Gene Tools by Syngene.

### **Statistical Analysis**

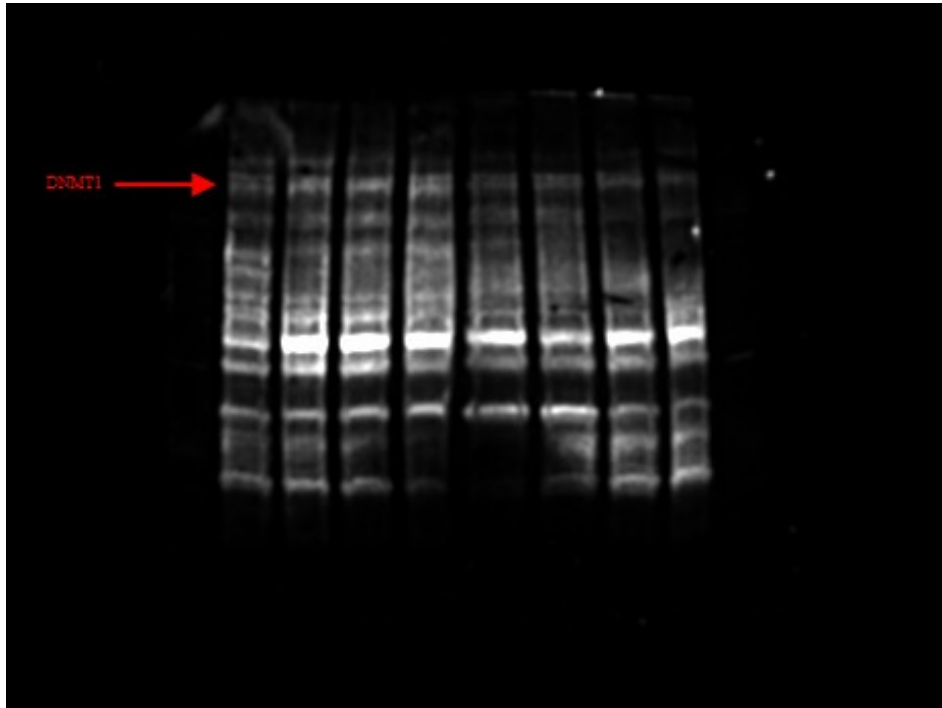
All data was analyzed using SPSS software (IBM Corporation, Armonk NY). For statistical analysis of DNMT1 and GR protein density involving only the NS and CS conditions, a Student's t-test was used to determine any differences between the two groups.

## RESULTS

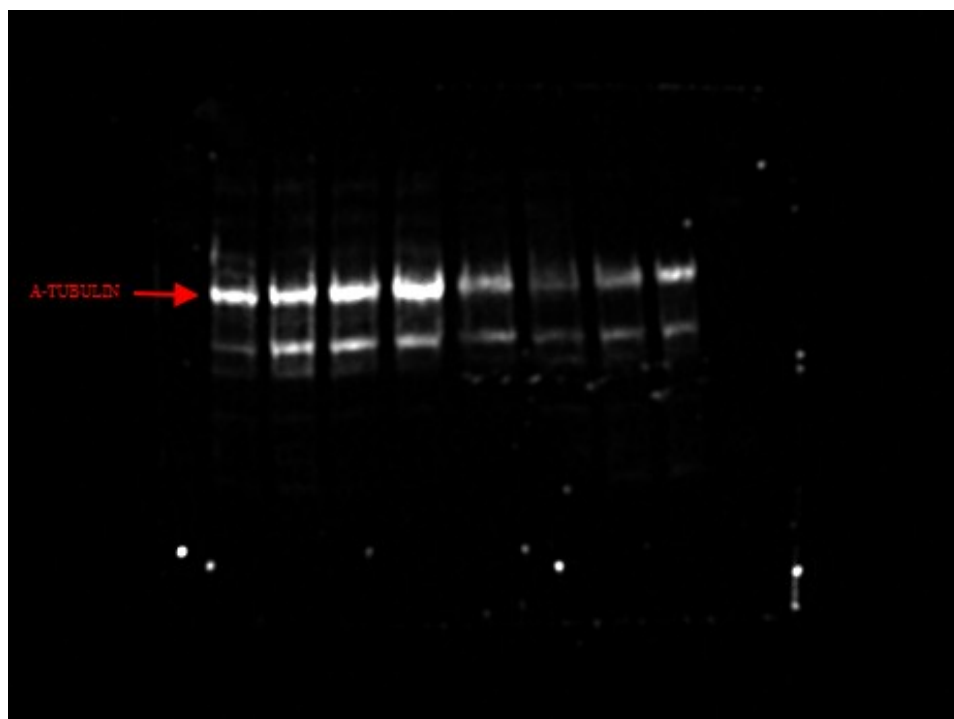
### Western Blot Imaging and Analysis

#### *DNMT1*

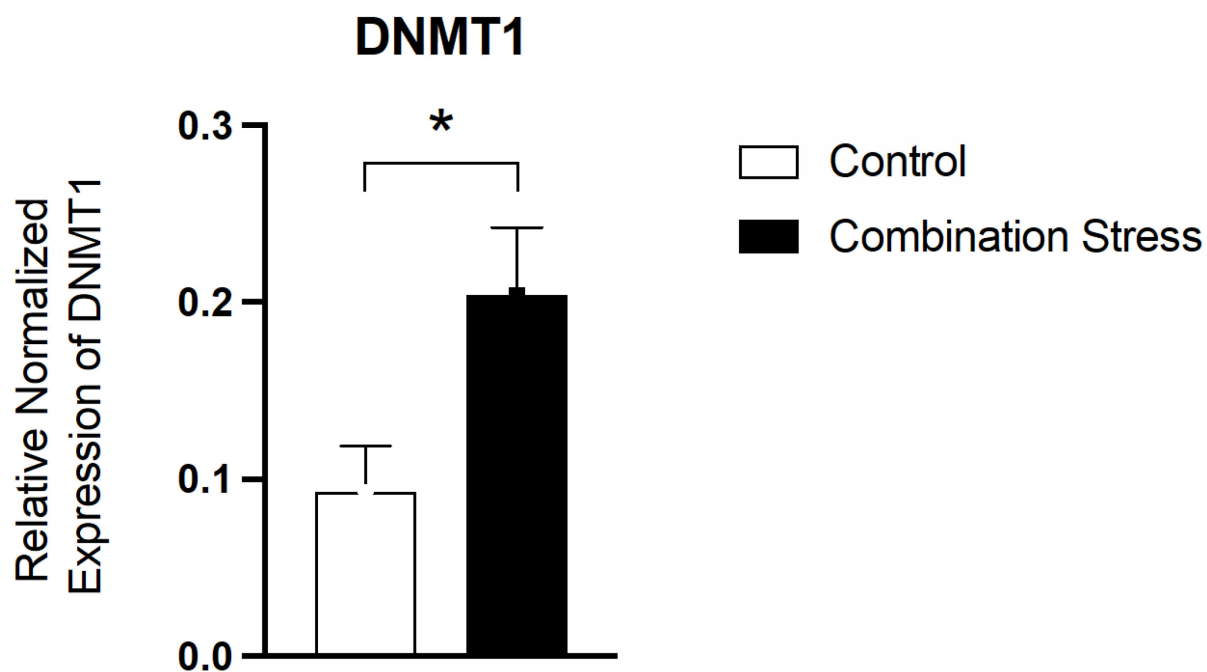
An independent samples *t*-test was performed to analyze the effects of pre- and postnatal stress on DNMT1 expression in the hippocampus. Results showed that mice that underwent the combination stress paradigm had significantly more DNMT1 compared to control mice that did not undergo the stress paradigm ( $t(13) = 2.461, p = 0.029$ ).



**Figure 2.** Image of Western Blot for DNMT1. The protein band is demarcated by the red arrow and runs at 183 kDa.



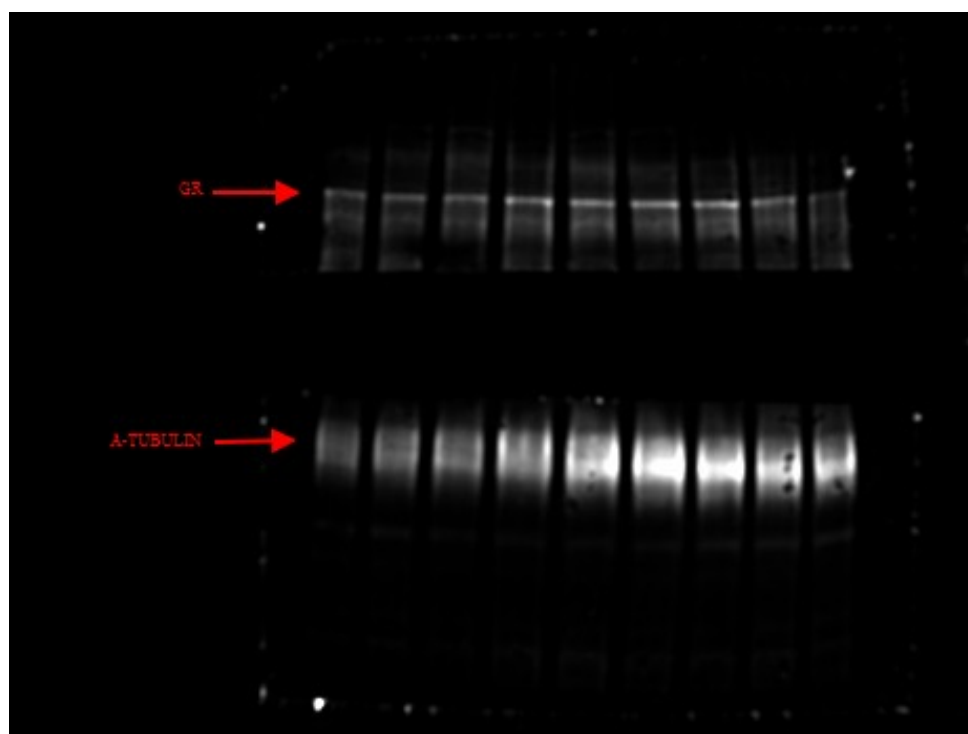
**Figure 3.** Image of Western Blot for  $\alpha$  – tubulin. The protein band is demarcated by the red arrow and runs at 50 kDa.



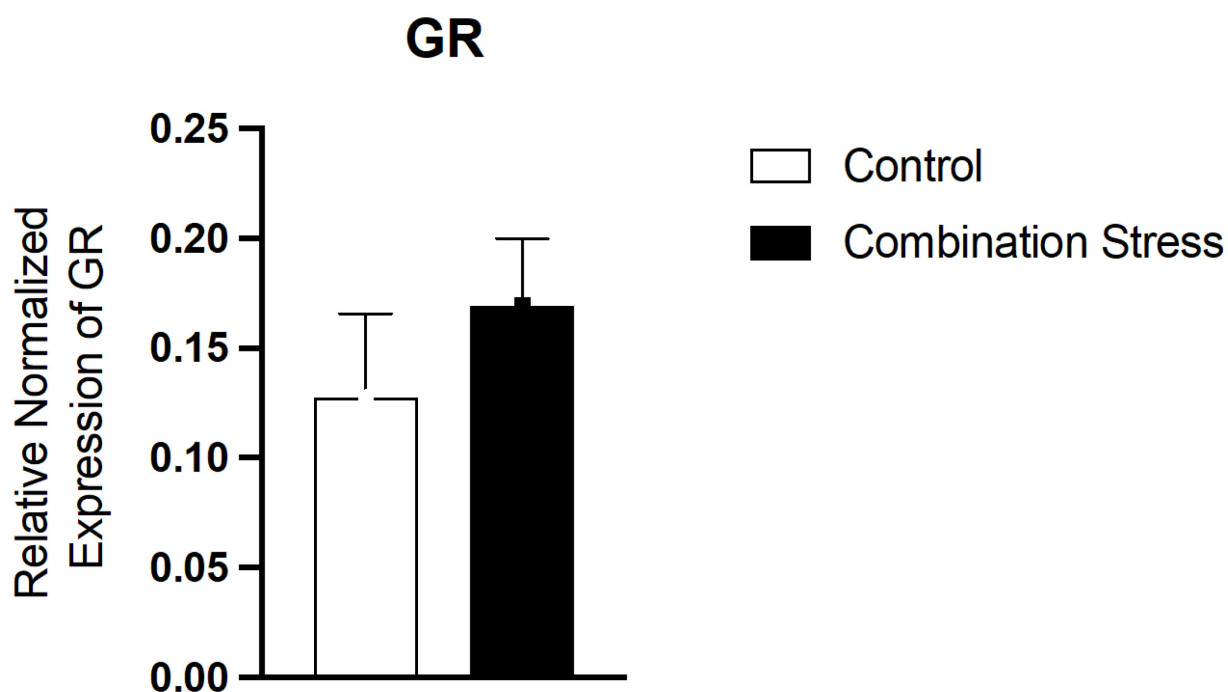
**Figure 4.** Results from Western Blot were normalized to  $\alpha$  – tubulin prior to being normalized to control group (No Stress/Saline). An independent samples *t*-test showed that mice that underwent the combination stress paradigm had significantly more DNMT1 compared to control mice. \* represents significant differences ( $p < 0.05$ ). Bars represent the mean  $\pm$  SEM.

### *Glucocorticoid Receptor*

To examine whether hippocampal glucocorticoid receptor (GR) expression was impacted by the combination stress paradigm, densitometry for the GR Western Blot was analyzed and another independent samples *t*-test was performed. Although mean GR expression was slightly higher in mice that underwent the combination stress paradigm compared to control mice, results revealed no significant difference between the groups ( $t(12) = 0.864, p = 0.404$ ).



**Figure 5.** Image of Western Blot for GR and  $\alpha$  – tubulin. The protein bands are demarcated by red arrows. GR is found at 97kDa and  $\alpha$  – tubulin is found at 50 kDa.



**Figure 4.** Results from Western Blot were normalized to  $\alpha$  – tubulin prior to being normalized to control group (No Stress/Saline). An independent samples *t*-test showed that mice that underwent the combination stress paradigm had no significant difference in GR expression compared to control mice. ( $t(12) = 0.864, p = 0.404$ ).

## DISCUSSION

The purpose of these experiments was to further understand the relationship between altered levels of pro-inflammatory cytokines in relation to levels of GR and DNMT1 in the hippocampus of mice exposed to prenatal and postnatal stress. This study used samples from mice who were exposed to an early bedding restriction paradigm, which has been shown to increase anxiety behaviors as well as have direct effects on cognition, learning, and memory (Walker et al, 2017). This stress administration model was used due to the ability for the stressor to be administered continuously throughout the prenatal and postnatal stress paradigm as opposed to during specific time periods.

Previous research in the lab investigated how ELS effects adulthood cytokine expression. Using RT-qPCR, the data showed a general downregulation in proinflammatory cytokines in the CS animals, but not in the NS animals. While IL-1 $\beta$  showed no significant differences between the CS and the NS animals, IL-6 and TNF- $\alpha$  showed downregulation with respect to cytokine expression. It is important to note that in the original study, a postnatal stress model was also used to assess changes in cytokine expression. Additionally, previous work in the lab utilized the administration of LPS to stimulate an acute inflammatory response shortly before collection of the hippocampal tissue. Data collected from these earlier manipulations show a generalized trend toward an increased inflammatory response in tissue collected after LPS injections across all three stress conditions, with significance seen between the LPS and saline model in IL-1 $\beta$ . These results substantiated previous research regarding an increased inflammatory response in the brain following the administration of LPS. Furthermore, postnatal stress animals were found to have significantly more mRNA than the combination stress animals, but neither condition was significant from the no stress condition. This data is consistent with the literature for postnatal stress, which has found that postnatal ELS can cause the exacerbation of the inflammatory response in the brain (Roque et al., 2016). In contrast, combination stress data suggests the opposite effect, with cytokine expression decreasing in these animals.

Further understanding the decrease in pro-inflammatory cytokine expression in the combination stress model is of notable interest. Deviation from the original hypothesis that combination stress would increase the inflammatory response suggests that stress during the prenatal period may override the effects that postnatal stress may have on adulthood inflammation. This claim is both refuted and substantiated in the literature, with one study showing increased basal expression of IL-1 $\beta$  and TNF- $\alpha$  in the hippocampus after prenatal stress

and others demonstrating immunosuppressive effects associated with prenatal stress (Diz-Chavez et al 2012, 2013, Pincus-Knackstedt et al 2006, Collier et al 2011). The latter studies found reductions in TNF- $\alpha$  expression in the periphery of females exposed to prenatal embryonic stress while the other study showed an alteration in the Th1/Th2 ratio in the blood of mice exposed to prenatal stress. This ratio shifted to favor Th2 cells (Pincus-Knackstedt et al 2006). This shift is important as Th1 and Th2 cells work in distinct and different immune response pathways where Th1 cells induce the cell-mediated immune response and secrete cytokines with pro-inflammatory properties like IL-2 and IFN- $\gamma$ , whereas Th2 cells play a large role in the allergic immune response and secrete IL-4 and IL-5 cytokines that have been noted for their immune regulatory qualities. The balance between these two cell types is important for the regulation of disease and inflammation as each cell type works in an antagonistic manner against the other (van Eden et al., 2002). For animals exposed to ELS, there has been a hypothesized link between prenatal stress and an increased Th2 cytokine profile in offspring, which mirrors that of the data seen in the CS mice. However, the mechanism by which the Th2 cells obtain dominance remains unclear. Certain evidence suggests that decreases in the hippocampal GR will result in decreased negative regulation of the HPA axis. This would result in an increase in cortisol production which has been shown to increase Th2 cytokine production (Al-Hussainy et al., 2020).

To determine if epigenetic modification to GR may have contributed to the alterations in the inflammatory response in CS animals, GR and DNMT1 were quantified through Western Blot analysis. As noted previously, decreases in hippocampal GR has been associated with decreased negative regulation of the HPA axis. Methylation by DNMT1 at this area could be one mechanism by which this downregulation and subsequent change in Th1/Th2 ratio occurs. Densitometry analysis and correction for the  $\alpha$  – tubulin loading control revealed no significant

difference in the density of GR in the hippocampus of the CS and NS models. These results differ from the hypothesis in which there was an expected decrease in GR for the CS model. However, for the densitometry analysis of DNMT1, there was a significant increase in the amount of DNMT1 in the CS hippocampus compared to the NS model. These findings are in alignment with the epigenetic modification theory in which increased stress results in an increase in epigenetic modification, one of which being DNA methylation.

These results give rise to two conclusions: 1) GR is likely not being methylated to a level that decreases protein expression 2) DNA methylation enzymes, and presumably DNA methylation, is occurring at higher rates in the CS model compared to the no stress control. While there has been evidence to suggest that methylation of the NR3C1 gene decreases hippocampal GR expression, there are many other potential targets for DNA methylation enzymes (Al-Hussainy et al., 2020). One such target is hypothesized to be BDNF, a neurotrophic factor that has been repeatedly shown to play a role in development, trophic support, and neuroplasticity (Heldt et al., 2007). RT-qPCR analysis in previous studies in the lab have shown decrease levels of BDNF in the hippocampus of mice exposed to ELS. Given this decrease, it is reasonable to assume a mechanism of the genomic silencing of BDNF expression by DNMT1. Other potential targets of DNMT1 may include mineralocorticoid receptor as well as genes that control cytokine production in the ELS offspring t-cells (Al-Hussainy et al., 2020). Methylation at either of these targets could explain the significant decrease in the level of pro-inflammatory cytokines in CS mice compared to the PS model. In either case, genomic sequencing techniques would be beneficial for understating which sites of the genome are being directly targeted by DNMT1 as a simple change in protein expression is not sufficient for understanding the mechanism behind the expression differences.

These findings give rise to many new questions surrounding the long-term effects of stress during early development. While epigenetics has been shown to play an important role in the programming of genomic modifications in adulthood, it is important to investigate the variations in epigenetic modification between genders in these ELS models. One study found data that suggested a strong sex differences in the reported consequences of ELS, with females being more resilient than males (Walker et al., 2017). Given the exclusion of females from this study, investigating these gender differences would be important to understanding the sexual dichotomy of the long-term effects of stress.

Overall, this study expands upon existing data demonstrating the negative effects that early life stress can have on the body while attempting to uncover a potential mechanism responsible for the inflammatory differences between CS and NS animal models. Understanding how stress impacts both the nervous and immune system is crucial for the prevention and treatment of diseases that affect our metabolism, psychology, cardiovascular pathology and much more. Through data collected in studies such as these, we hope to one day provide the knowledge that will lead to new methods of dealing and protecting against stress.

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