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Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex

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Abstract

Quality of maternal care experienced during infancy is a key factor that can confer vulnerability or resilience to psychiatric disorders later in life. Research continues to indicate that early-life experiences can affect developmental trajectories through epigenetic alterations capable of affecting gene regulation and neural plasticity. Previously, our lab has shown that experiences within an adverse caregiving environment (i.e. maltreatment) produce aberrant DNA methylation patterns at various gene loci in the medial prefrontal cortex (mPFC) of developing and adult rats. This study aimed to determine whether caregiver maltreatment likewise affects expression levels of several genes important in regulating DNA methylation patterns (*Dnmt1*, *Dnmt3a*, *MeCP2*, *Gadd45b*, and *Hdac1*). While we observed minimal changes in gene expression within the mPFC of developing rats, we observed expression changes for all genes in adult animals. Specifically, exposure to maltreatment produced a significant decrease in mRNA levels of all epigenetic regulators in adult males and a significant decrease in *Gadd45b* in adult females. Our results here provide further empirical support for the long-term and sex-specific epigenetic consequences of caregiver maltreatment on the mPFC.

Keywords

epigenetics; early-life stress; maternal care; mPFC; *Dnmt*; *Hdac*; *Gadd45b*; *MeCP2*

1. Introduction

Infancy is a sensitive period of development in which alterations in the quality or quantity of maternal care are capable of producing dramatic effects on neurobiological and behavioral outcomes (Caldji et al., 2000; Cicchetti and Toth, 2005; Sanchez, 2006). Mechanisms underlying these environmentally-driven phenomena are not fully understood, but over the past decade the birth of epigenetics research has caused a revolution in our understanding of gene-environment interactions in this capacity in the developing and adult mammalian brain. Although epigenetic modifications were originally thought to only program patterns of gene expression during cellular development and differentiation, a significant body of research now indicates that such modifications occur in response to environmental signals from infancy to senescence, with significance for long-lasting changes in gene regulation and expression (Caldji et al., 2011; Roth, 2012) and neuronal plasticity (Day and Sweatt, 2011).

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Various types of epigenetic modifications have been identified, such as DNA methylation and post-translational modifications to histone tails (i.e. acetylation and methylation), and together they help determine whether DNA is accessible for gene transcription (Strahl and Allis, 2000). Of these modifications, DNA methylation and histone acetylation have been the most extensively studied in the context of behavior, and are driven and maintained by various enzymes and proteins. DNA methylation, for example, is catalyzed by a family of DNA methyltransferases (DNMTs) that includes DNMT1 and DNMT3a (Moore et al., 2012). DNMT1 is a maintenance methyltransferase which is responsible for adding methyl groups to the complimentary strand of hemimethylated DNA, thereby maintaining DNA methylation patterns during the DNA replication process. DNMT3a is a *de novo* methyltransferase, capable of catalyzing new methylation patterns. While both *Dnmt1* and *Dnmt3a* mRNA levels are highest in the mammalian cortex, hippocampus, striatum, and cerebellum during late prenatal and early postnatal development, both enzymes are present and functional throughout the lifetime (Feng et al., 2005; Goto et al., 1994; Inano et al., 2000; Matrisciano et al., 2012; Miller et al., 2012; Siegmund et al., 2007). *Dnmt3a* mRNA, for example, is at high levels within the rodent mPFC for the first three weeks of life but dramatically decreases to adult levels by postnatal day (PN) 21 (Miller et al., 2012). *Dnmt1* mRNA levels within the rodent amygdala and subregions of the hippocampus are down-regulated within the first postnatal week (Simmons et al., 2012).

Another protein associated with DNA methylation is Methyl-CpG Binding Protein-2 (MeCP2), which binds to methylated cytosines and recruits either histone deacetylases (HDACs) and other corepressors to suppress gene transcription (Jones et al., 1998) or CREB1 and other coactivators to promote gene transcription (Chahrour et al., 2008; Uchida et al., 2011). A deficiency in MeCP2 has been widely associated with Rett syndrome, a neurodevelopmental disorder causing mental retardation with early onset in childhood, and MeCP2 mutant mice have been shown to exhibit many of the same cognitive deficits and neuroanatomical abnormalities associated with Rett syndrome (Chen et al., 2001; Jorgensen and Bird, 2002; Stearns et al., 2007). These studies have also emphasized the importance of MeCP2 in the developing and mature brain, with prefrontal cortical expression of this protein present during early fetal stages and increasing until late childhood when levels are maintained into adulthood (Akbarian et al., 2001; Balmer et al., 2003; Kaufmann et al., 2005; Shahbazian et al., 2002).

Recently, investigators have discovered that the Growth Arrest and DNA-Damage-Inducible beta (Gadd45b) protein plays an important role in DNA demethylation (i.e. the active removal of methyl groups). Instead of breaking the strong covalent bonds between methyl groups and cytosines, Gadd45b instead functions through a DNA-repair-like mechanism, in which an unmethylated cytosine replaces the methylated cytosine after a sequence of molecular events (Ma et al., 2009a; Ma et al., 2009b). The ontogenetic profile of Gadd45b has not been characterized, but recent work has demonstrated its role in regulation of memory formation (Leach et al., 2012; Sultan et al., 2012). Additional work in patients with psychosis has shown an increase in parietal cortical levels of *Gadd45b* mRNA and protein in comparison to healthy controls, demonstrating its possible contributory role to major psychosis (Gavin et al., 2012).

While the previously mentioned proteins and enzymes work to regulate DNA methylation patterns, the ability of histones to further regulate transcription adds another layer of complexity. The addition of an acetyl group to a lysine residue of the N-terminal histone tail is able to neutralize the positive charge and loosen the chromatin complex, allowing for increased binding of transcription factors and therefore increased gene expression. This process is catalyzed by histone acetyltransferases (HATs) and reversed by histone deacetylases (HDACs). HDACs are present throughout the lifetime, and the majority of

these enzymes show changes in mRNA levels throughout development and adulthood (Levine et al., 2012). For example, while *Hdac1* mRNA levels are relatively stable between PN21, PN28, and PN60 in the forebrain neocortex of mice, *Hdac3* mRNA show a dramatic increase between PN28 and 60 (Levine et al., 2012) Alterations in mRNA levels of a number of HDACs have been implicated in psychiatric disorders (Sharma et al., 2008) and the lasting effects of early-life stress (Levine et al., 2012).

Previously, we found that exposing infant rats to an adverse caregiving environment produces DNA methylation alterations that are present in both the developing and adult whole (Roth et al., 2009) and medial (Blaze et al., 2013) prefrontal cortex. While our 2009 study measured methylation in the prefrontal cortex as a whole, the importance of the medial prefrontal cortex (mPFC) in developmental trajectories associated with early-life stress is beginning to be highlighted in the literature. For example, this region has long been recognized for its role in mediating the stress response (Diorio et al., 1993), and various groups have recently demonstrated changes in mPFC gene expression (Uchida et al., 2010) and structural abnormalities (Spinelli et al., 2009) following early-life stress. In our 2013 study, we found sex-specific alterations in methylation of the *Brain-derived neurotrophic factor* (*Bdnf*) gene in the mPFC of adolescent and adult rats that experienced early-life maltreatment. Additionally, differences in *reelin* methylation were only present in adulthood for maltreated males and both males and females exposed to nurturing care outside the home cage (Blaze et al., 2013). The aim of this study was to determine whether caregiver maltreatment likewise affects expression levels of several genes important in regulating epigenetic patterns. Here we examined whether caregiver maltreatment altered *Dnmt1, Dnmt3a, MeCP2, Gadd45b,* or *Hdac1* mRNA levels in the developing and adult mPFC.

2. Materials and Methods

2.1 Subjects

All procedures were approved by the University of Delaware Animal Care and Use Committee. Brain tissue came from a total of twenty-two litters for all age cohorts combined, and this sample matches that previously used (Blaze et al., 2013). To generate subjects, male and female outbred Long-Evans rats from Harlan were housed in our temperature and light-controlled (12-hours light/dark cycle, lights on at 6:00am) colony room in standard polypropylene cages and given ample bedding material. Behavioral manipulations were all performed during the light cycle, and animals were provided with food and water *ad libitum*. Females were bred with males in our breeding colony and allowed to raise at least one litter of pups before the start of the experiment to eliminate confounds associated with first-time mothers. Dams and pups were left undisturbed on PN 0 (day of pup birth) and litters were culled to approximately 6 males and 6 females per litter on PN1.

2.2 Caregiving Manipulations

Our caregiving manipulations are based on a method previously reported (Blaze et al., 2013; Roth et al., 2009) and adapted from earlier studies (Ivy et al., 2008; Roth and Sullivan, 2005). On PN1, we used a within-litter design and split a litter into three equal groups for our three treatment conditions. For the first seven days of life (PN1-7), one group of pups (normally 2 females and 2 males) was exposed to a stressed, non-biological caregiver for thirty minutes each day (maltreatment condition). To produce maltreatment behaviors toward infants, the dam was provided insufficient nesting material (100 ml of wood shavings) while she was in a novel environment. Littermates were exposed to a nurturing caregiver outside of the home cage (cross-foster care condition), which consisted of another non-biological lactating dam who was allowed one hour to habituate to her new environment

and given sufficient nesting material (approximately 2 cm layer of wood shavings). Both maltreatment and cross-foster care chambers were maintained between 24-29°C. Additional littermates were quickly marked for identification and weighed at the beginning of each session and immediately returned to the biological mother in the homecage (normal maternal care condition). Pups in all conditions were marked with the same non-toxic permanent marker for identification. After each day's thirty minute session, maltreatment and cross-foster care pups were returned to the homecage with the biological mother and all litters remained undisturbed except for weekly cage changes and treatment sessions. For our PN8 time-point, pups were sacrificed 24 hours after the last caregiving condition, but for older time-points pups were weaned at PN21 and housed in same-sex/treatment pairs until sacrifice in adolescence (PN30) or adulthood (PN90).

Maternal caregiving behaviors were scored via live observation or video recording by two trained observers. For each litter, maltreatment or nurturing behaviors were tallied in five-minute time bends and averaged across all seven exposure days. To measure pup response to maternal behaviors, we measured pup audible and 40 kHz ultrasonic vocalizations (Batbox III D, NHBS Ltd., UK) by marking the presence of a vocalization in each one-minute time bend and averaging across the seven exposure days. As previously reported for this cohort of rats (Blaze et al., 2013), we observed significant levels of aversive caregiving towards infants (stepped on, dropped during transport, dragged while nipple attached, actively avoided, and roughly handled infants) within the maltreatment condition, but similar high levels of nurturing care (infant licking, grooming, and nursing) within the cross-foster and normal care conditions. Furthermore, we observed high amounts of audible and ultrasonic distress vocalizations emitted within our maltreatment condition but not within the environments where pups experienced nurturing care (our normal and cross-foster groups).

2.3 Gene Expression Assays

Tissue was collected from animals at baseline conditions at PN8, PN30, or PN90. Brains were removed, sliced using a 1 mm brain matrix, flash frozen on untreated slides with 2-methylbutane, and placed in a -80 freezer until later processing. The mPFC (consisting of bilateral prelimbic and infralimbic tissue) was dissected on dry ice using stereotaxic coordinates and RNA was extracted (Qiagen Inc., Valencia, CA). Quantification and assessment of nucleic acid quality were determined using spectrophotometry (NanoDrop 2000). Reverse transcription was performed using a cDNA synthesis kit (Qiagen) on RNA, and cDNA was amplified by real-time PCR (Bio-Rad CFX96) with Taqman probes (Applied Biosystems) to target *Dnmt1*, *Dnmt3a*, *MeCP2*, *Gadd45b*, *Hdac1*, or *tubulin* (for a reference gene) mRNA. All reactions for each gene in the expression assays were run in triplicate. Product specificity was determined by gel electrophoresis.

2.4 Statistical Analyses

For gene expression assays, we used the comparative Ct method to obtain the relative fold change for experimental (maltreatment or cross-foster) vs. control (normal care) groups (Livak and Schmittgen, 2001). We used one-sample t-tests to compare fold change differences between experimental and control groups. Two-way ANOVAs were used to analyze differences across treatment groups and sex and were followed by Bonferroni post hoc tests if appropriate. Differences were considered to be statistically significant for $p<0.05$.

3. Results

3.1. Experiment 1: Infant Gene Expression

At PN8, brains were removed 24 hours after the last caregiving manipulation to assess whether mRNA levels of several epigenetic regulators within the mPFC had been affected by repeated exposures to our various caregiving conditions (Figure 1a-e). No significant differences were detected between our experimental and controls groups for *Dnmt1*, *MeCP2*, *Gadd45b*, or *Hdac1* (one-sample t-tests, all p's>0.05). Likewise, no differences were detected between our experimental groups for these same gene loci (two-way ANOVAs, all p's>0.05). A one-sample t-test however revealed that females who experienced nurturing care outside the homecage had significantly less *Dnmt3a* mRNA compared to same sex normal care controls (t_8=2.85, p<0.05) (Figure 1b). Likewise, maltreated-females had a trending decrease in *Dnmt*3a mRNA (t_9=2.06, p=0.07). Additionally, a two-way ANOVA for this gene locus showed a significant main effect of sex (F (1,34)=5.85, p<0.05) but no main effect of infant condition or an infant condition by sex interaction.

3.2 Experiment 2: Adolescent Gene Expression

Another cohort of animals was sacrificed at PN30 to assess adolescent levels of epigenetic regulators within the mPFC (Figure 2a-e). At this time point, no significant differences were detected in mRNA levels for *Dnmt1*, *Dnmt3a*, *Gadd45b*, or *Hdac1* (one-sample and two-way ANOVAs, all p's>0.05). For *MeCP2* however, maltreated males had significantly less mRNA in comparison to normal care (t_7=5.941, p<0.001) and cross-foster care controls (t_{15}=2.54, p<0.05) (Figure 2c). A two-way ANOVA for this gene locus revealed a trending effect of sex (F(1,29)=3.73, p=0.06), and no main effect of infant condition or an interaction effect (p's>0.05).

3.3 Experiment 3: Adult Gene Expression

mPFC mRNA levels were likewise assessed on another cohort allowed to reach adulthood (PN90). Unlike our other two age groups, we observed significant group differences at all gene loci in our adult animals (Figure 3a-e). Males who experienced maltreatment had less *Dnmt1* (t_{13}=2.629, p<0.05) and *MeCP2* (t_{13}=4.689, p<0.001) mRNA compared to normal controls (Figure 3, panels a and c). Two-way ANOVAs for *Dnmt1* and *MeCP2* revealed no other significant differences (p's>0.05). Maltreated males also had less *Dnmt3a* mRNA in comparison to normal care controls (t_{13}=3.934, p<0.01) (Figure 3b). In addition, a two-way ANOVA showed a main effect of infant condition (F(1,37)=12.41, p=0.001), with further analyses showing that both maltreated males (t_{22}=2.703, p=0.01) and females (t_{15}=2.269, p<0.05) had significantly less *Dnmt3a* mRNA compared to animals that experienced nurturing care outside the home cage (cross-foster care condition).

For *Gadd45b*, maltreated males again had significantly less mRNA in comparison to normal care controls (t_{12}=5.917, p<0.001), and maltreated females likewise had lower *Gadd45b* mRNA levels than controls (t_{10}=4.202, p<0.01) (Figure 3d). A two-way ANOVA revealed a main effect of sex (F(1,35)=7.248, p=0.01), infant condition (F(1,35)=23.80, p<0.0001), and a sex by infant condition interaction (F(1,35)=3.965, p=0.05). Post-hoc analyses showed that maltreated males (t_{21}=2.384, p<0.05) and females (t_{14}=4.262, p<0.001) had less *Gadd45b* mRNA than sex-matched animals from the cross-foster care group. Additionally, females that experienced nurturing care outside of the home cage had significantly more *Gadd45b* mRNA than cross-foster males (t_{13}=2.290, p<0.05). Finally, maltreated males had significantly lower *Hdac1* mRNA levels than normal care controls (t12=4.354, p<0.001) (Figure 3e). A two-way ANOVA revealed a main effect of infant condition (F(1,37)=7.313, p=0.01) for *Hdac1* mRNA, but no main effect of sex or a sex by condition interaction (p's>0.05).

4. Discussion

Using this rodent model, we have previously characterized the effects of repeated exposure to maltreatment or nurturing care during the first postnatal week on *Bdnf* and *reelin* DNA methylation patterns in the developing and adult mPFC (Blaze et al., 2013). Specifically, in adulthood, both males and females who experienced maltreatment in infancy displayed decreased methylation of DNA associated with *Bdnf* exon I. Maltreated-females also had increased methylation of *Bdnf* exon IV relative to normal and cross-foster care controls and maltreated-males. These group differences in methylation were not present in adolescence or infancy. Instead, a different pattern was present in adolescence, with maltreated males showing increased methylation at *Bdnf* exon I and females showing a decrease in methylation at exon IV relative to normal care controls. At the *reelin* promoter, we observed greater methylation in both adult males that were maltreated and nurtured outside of the homecage. Similar to our *Bdnf* effects, these group differences were not present earlier in development.

In the current study, we sought to determine the effects of these caregiving environments on genes important for establishing, maintaining, or reversing DNA methylation patterns. In the adult (PN90) mPFC, we found that maltreated-males exhibited less *Dnmt1*, *Dnmt3a*, *MeCP2*, *Gadd45b*, and *Hdac1* mRNA than normal care controls. Various groups have demonstrated alterations in DNMT1 and DNMT3a mRNA or protein levels as the result of prenatal stress (Jensen Peña et al., 2012; Matrisciano et al., 2012). Though offspring of prenatally stressed mothers have been found to have increased *Dnmt1* (cortex) and *Dnmt3a* (placenta) mRNA levels (Jensen Peña et al., 2012) and increased DNMT1 protein levels in adult brain tissue (Matrisciano et al., 2012), we found here decreased *Dnmt1* and *Dnmt3a* mRNA levels in males who experienced maltreatment during the first seven postnatal days. This could demonstrate the differential sensitivity of these regulators to environmental factors during prenatal versus postnatal periods.

Maltreated-males also showed a change in *MeCP2* expression, while female *MeCP2* mRNA levels remained similar to that of controls. Transient sex differences in *MeCP2* have previously been observed in the healthy amygdala and ventromedial hypothalamus, such that males had less *MeCP2* mRNA and protein at PN1 but the difference was no longer present at PN10 (Kurian et al., 2007). It has also been shown that males with decreased MeCP2 protein (but not female) expression exhibit reductions in juvenile social play behavior (Kurian et al., 2008; McCarthy et al., 2009; Nagarajan et al., 2006). Our data here are consistent with other reports demonstrating the ability of repeated bouts of maternal separation to have lasting effects on MeCp2. For example, one report has shown reduced binding of MeCP2 in the hypothalamus of separated mice (Murgatroyd et al., 2009), and another more recent report has shown a reduction in MeCP2 cell counts in the adult nucleus accumbens of separated rats (Lewis et al., 2013). We also found changes at the *Gadd45b* gene locus, a recently identified candidate for active DNA demethylation (Ma et al., 2009a). To our knowledge, no studies to date have investigated the developmental profile of *Gadd45b* mRNA or protein levels, or the effects of early-life stress on this gene. In our study, both adult males and females with a history of maltreatment showed decreased levels of *Gadd45b*. While adult expression levels for all other epigenetic regulators that we examined were only lower in maltreated-males, this was the only locus reaching statistical significance in maltreated-females.

We also found a decrease in *Hdac1* mRNA in males with a history of maltreatment. While the effects of early-life stress on DNA methylation have been widely studied by our lab and others, the effect of early-life stress on histone modifications is a less common area of research though these two mechanisms (histone modifications and DNA methylation) are

not mutually exclusive. HDACs can participate in gene silencing by their binding to MeCP2 and working in parallel with other recruited corepressors. Adult offspring of low licking/grooming mothers have been shown to have decreased H3K9 acetylation in the hippocampus, which could be reversed with the HDAC inhibitor trichostatin-A (Weaver et al., 2004). Maternal separation during infancy has also been shown to produce changes in histone acetylation and methylation within the developing and adult hippocampus (Suri et al., 2013; Xie et al., 2013). A recent study revealed decreased *Hdac1* mRNA in the forebrain neocortex of adult male mice that had experienced maternal separation in infancy (Levine et al., 2012), which parallels our findings. Their study also showed the capacity of maternal separation to alter mRNA levels of other classes of HDAC enzymes, suggesting it would be worthwhile to also characterize changes for additional HDACs in our model.

While the observed gene expression changes were robust in adulthood, several months post-manipulation, only few gene expression changes were present earlier in development. At PN30 (adolescence), maltreated-males showed a decrease in *MeCP2* mRNA relative to normal and cross-foster controls. In infants, 24 hours after the last caregiving exposure, the only significant difference observed was a decrease in *Dnmt3a* mRNA levels in females who had experienced nurturing care outside of the homecage. Maltreated-females also showed a trending decrease in *Dnmt3a* levels. As previously mentioned, *Dnmt3a* is a *de novo* methyltransferase that establishes new methylation patterns, and this enzyme exhibits the highest mRNA levels during the first three weeks of life (Miller et al., 2012). There is also evidence that females have higher levels during the first two postnatal weeks than males (Jessen and Auger, 2011; Kolodkin and Auger, 2011). Our observation of a sex difference in *Dnmt3a* mRNA that is present at PN8 but no longer present at our adolescent time point may be in line with these sex and age effects. Furthermore, our data illustrate the sensitivity of *Dnmt3a* to changes in the caregiving environment.

A common theme among our observations from this study was aberrant gene expression specific to our maltreatment group, especially for males. While several of these genes are known to exhibit sexually-dimorphic expression patterns that could contribute to our group differences, it is possible that differences in maternal care toward male and female pups both within the homecage (before and after treatment) and during our manipulations contributed to gene differences. Though the design of the present experiment did not allow us to distinguish caregiving behaviors directed at males versus those at females, it is known that males are licked more by the dam within the nest (Hao et al., 2011; Moore and Morelli, 1979). Regardless of a mechanism of change, our data are consistent with the notion that there could be large-scale (global) methylation changes in our adult animals, especially males. Using the same paradigm, our previous work has shown sex-specific alterations in *Bdnf* DNA methylation levels in adulthood (Blaze et al., 2013), and other work is also in line with the idea of sexually-dimorphic DNA methylation changes throughout the lifespan (Schwarz et al., 2010). Future work will be necessary to identify whether other genes show similar experience-induced changes in DNA methylation, and to co-localize changes in DNMT, MeCP2, Gadd45b, and HDAC binding to specific gene loci.

A second common theme that continues to be present with our work is epigenetic differences that vary across or emerge later in development for our maltreatment group. This could be partially attributed to the late developing nature of the medial prefrontal cortex itself, with connections and circuits continuing to mature well past the postnatal period and into adolescence (Benes et al., 2000). It is also known that some of the enzymes/proteins measured as markers of epigenetic regulation are involved in neuronal plasticity (Ballas et al., 2005; Ma et al., 2009b), which may be potentiated at later developmental time points. Another factor that may be responsible for these observations is that epigenetic patterns (both DNA methylation and gene expression levels of the regulators we examined) are

known to naturally change over the course of the lifespan in “normal” animals, thus our baseline (i.e. levels within the normal maternal care group) to which we compare our experimental groups is presumed to be changing too with age (Lister et al., 2013; Numata et al., 2012; Simmons et al., 2012). Finally, it is possible that social factors experienced later in life (i.e. experiences with cagemates after weaning) also contributed to the observed gene profiles in the maltreated-animals.

While our assays failed to detect significant gene expression changes for many of our regulators in infancy or adolescence, it is possible that our global approach missed changes that occurred at the level of specific genes or even within specific cell-types. For example, it has been observed that patterns of DNA methylation in the frontal cortex can depend on cell-type (Shulha et al., 2013), suggesting that expression levels of epigenetic regulators could differ between glia and neurons. While the techniques used here limit us from concluding anything about such changes in these tissue samples, it is a future avenue of research in our lab. Future research should also address the degree to which these differences in mRNA translate into differences in protein expression.

In conclusion, while it is clear that childhood maltreatment poses a significant risk for psychopathology, the biological basis of this phenomenon remains an important topic for investigation. Using our rodent model, we have shown that repeated exposure to caregiver maltreatment during the first postnatal week has the capacity to alter DNA methylation. Here we extend this work to show that postnatal maltreatment likewise alters expression levels of genes important for establishing, maintaining, or reversing DNA methylation. Additional studies are necessary to link these gene methylation and expression changes with histone alterations, protein expression, subsequent behavioral outcomes, and to explore their reversibility.

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Abbreviations

mPFC	medial prefrontal cortex
DNMT	DNA methyltransferase
MeCP2	Methyl-CpG Binding Protein-2
Gadd45b	Growth Arrest and DNA-Damage-Inducible beta
HAT	histone acetyltransferase
HDAC	histone deacetylase
PN	postnatal day

Bdnf	brain-derived neurotrophic factor

Highlights

- We assessed the impact of early adversity on expression of epigenetic regulators (fold changes in mRNA).
- Minimal fold changes were detected within the infant and adolescent mPFC.
- Adult males and females who experienced maltreatment displayed significant fold changes.
- Data indicate long-term, sex-specific epigenetic changes after early adversity.

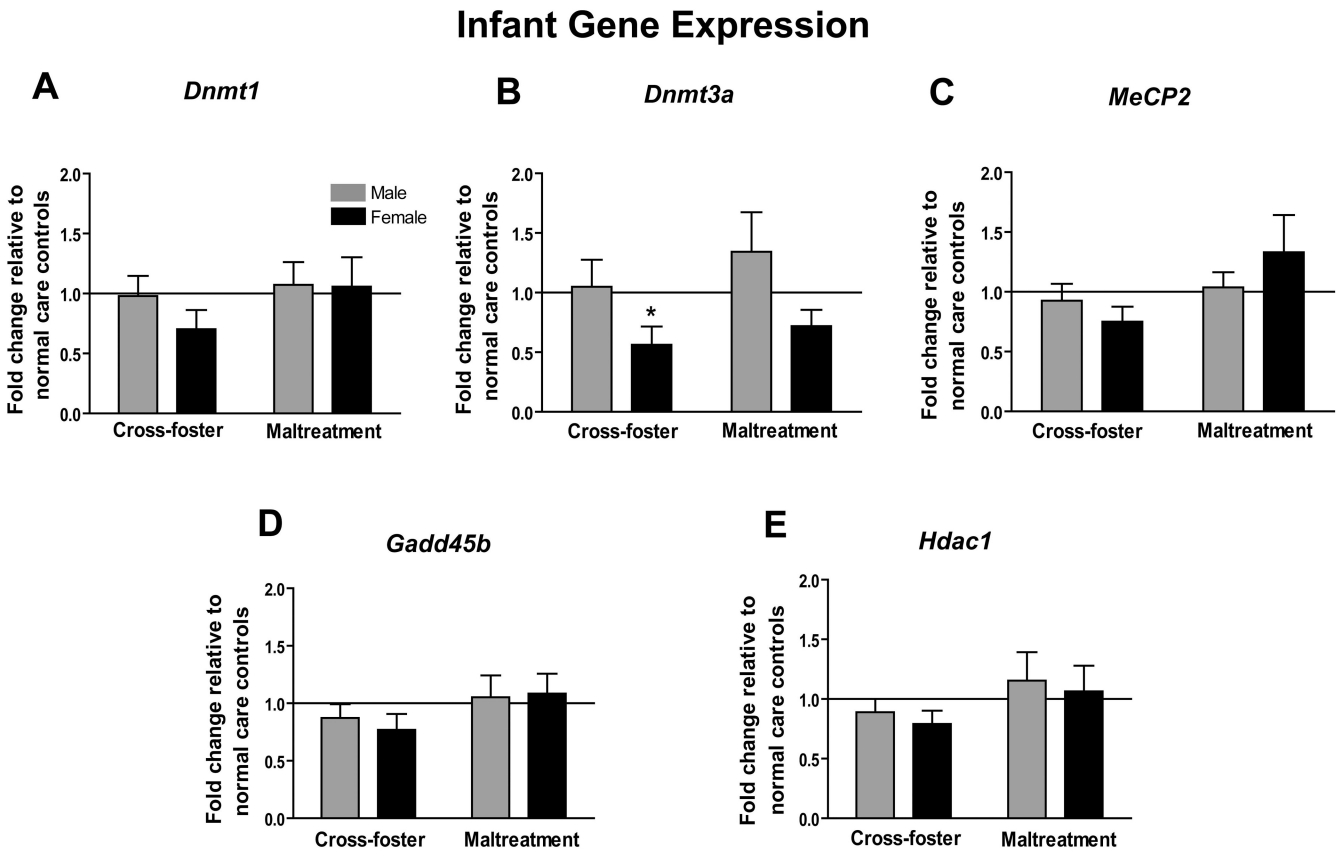

Figure 1.

Infant (PN8) mPFC mRNA expression for epigenetic regulator genes (**a**) *Dnmt1* (**b**) *Dnmt3a* (**c**) *MeCP2* **(d)** *Gadd45b* **(e)** *Hdac1*. (for all genes- n=9-10 per group; *p<0.05 versus normal care controls; error bars represent SEM)

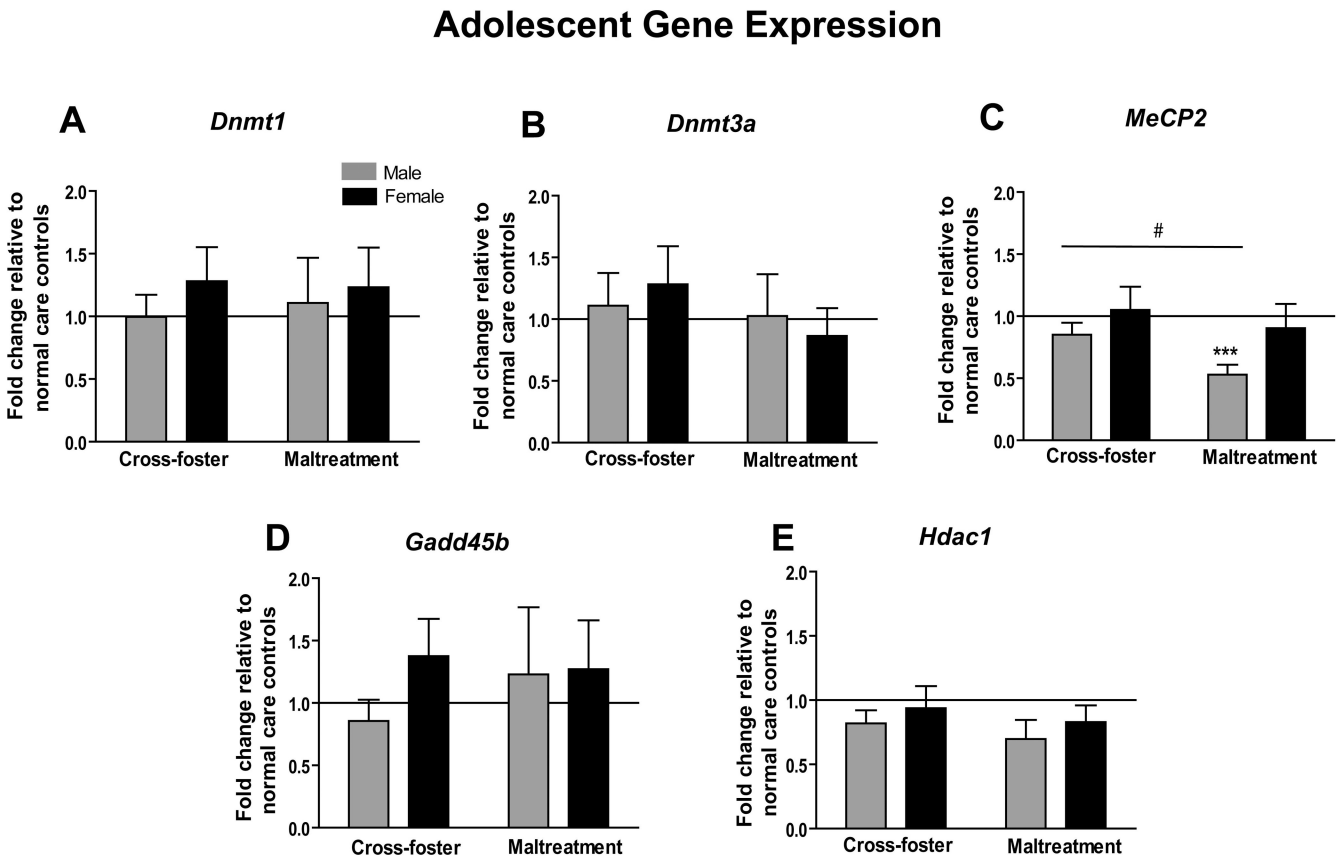

Figure 2.

Adolescent (PN30) mPFC mRNA expression for epigenetic regulator genes (**a**) *Dnmt1* (**b**) *Dnmt3a* (**c**) *MeCP2* **(d)** *Gadd45b* **(e)** *Hdac1*. (n=7-9 per group; ***p<0.001 versus normal care controls; #p<0.05 between groups; error bars represent SEM)

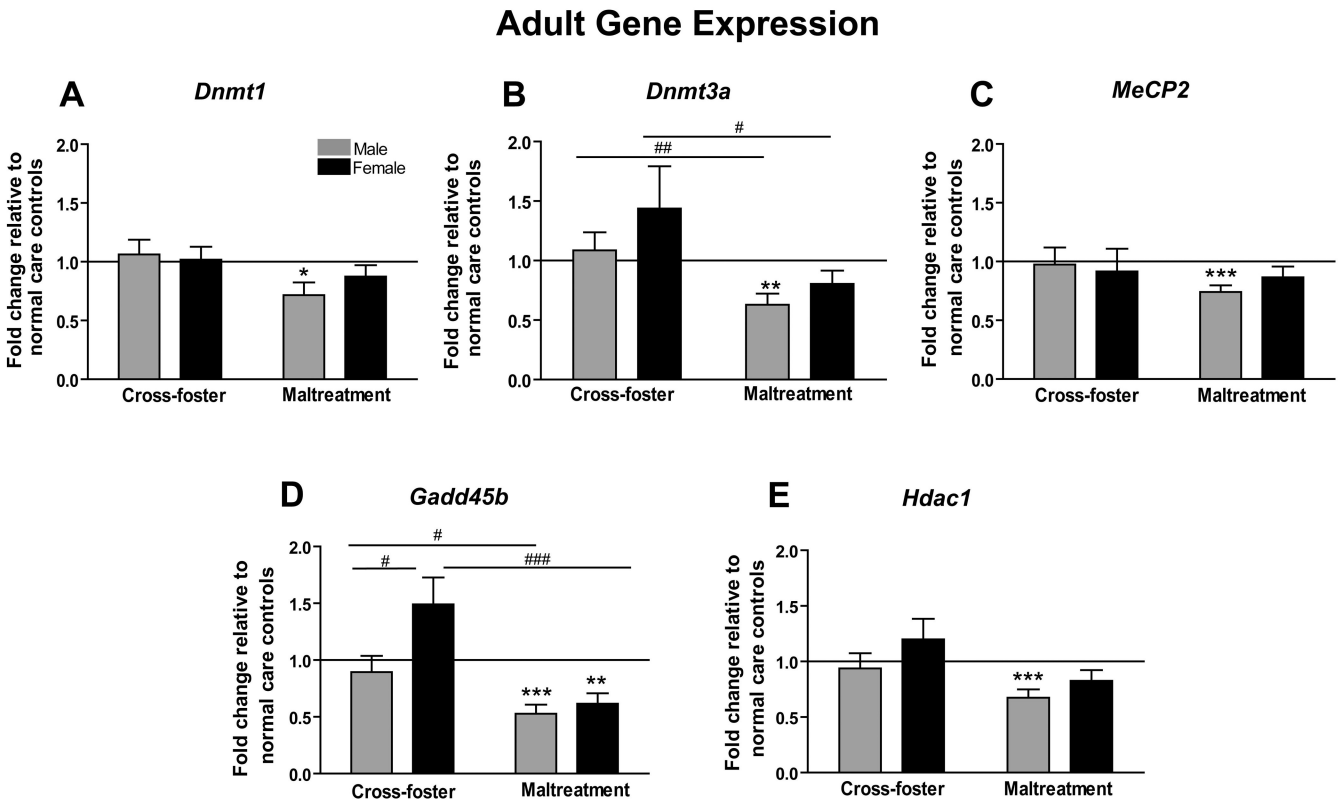

Figure 3.

Adult (PN90) mPFC mRNA expression for epigenetic regulator genes (**a**) *Dnmt1* (**b**) *Dnmt3a* (**c**) *MeCP2* **(d)** *Gadd45b* **(e)** *Hdac1*. (for all genes- n=5-6 cross-foster care females, n=10 cross-foster care males, n=12 maltreatment females, n=10 maltreatment males; *p<0.05, **p<0.01, ***p<0.001 versus normal care controls; $^{\#}$p<0.05 between sexes; $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 between groups; error bars represent SEM)