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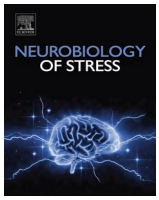

Maternal immune activation alters placental histone-3 lysine-9 tri-methylation, offspring sensorimotor processing, and hypothalamic transposable element expression in a sex-specific manner

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ABSTRACT

Animal models of maternal immune activation (MIA) are central to identifying the biological mechanisms that underly the association between prenatal infection and neuropsychiatric disorder susceptibility. Many studies, however, have limited their scope to protein coding genes and their role in mediating this inherent risk, while much less attention has been directed towards exploring the roles of the epigenome and transposable elements (TEs). In Experiment 1, we demonstrate the ability of MIA to alter the chromatin landscape of the placenta. We induced MIA by injecting 200 μg/kg (i.p.) of lipopolysaccharide (LPS) on gestational day 15 in Sprague-Dawley rats. We found a sex-specific rearrangement of heterochromatin 24-h after exposure to MIA, as evidenced by an increase in histone-3 lysine-9 trimethylation (H3K9me3). In Experiment 2, MIA was associated with long-term sensorimotor processing deficits as indicated by reduced prepulse inhibition (PPI) of the acoustic startle reflex in adult male and female offspring and an increased mechanical allodynia threshold in males. Analyses of gene expression within the hypothalamus-chosen for its involvement in the sex-specific pathogenesis of schizophrenia and the stress response-revealed significantly higher levels of the stress-sensitive genes *Gr* and *Fkbp5*. Deleterious TE expression is often a hallmark of neuropsychiatric disease and we found sex-specific increases in the expression of several TEs including IAP, B2 SINE, and LINE-1 ORF1. The data from this study warrant the future consideration of chromatin stability and TEs as part of the mechanism that drives MIA-associated changes in the brain and behavior.

1. Introduction

Epidemiological studies have demonstrated a link between gestational exposure to infection and an increased risk for developing neuropsychiatric disorders such as autism and schizophrenia (Brown et al., 2014; Meyer, 2019; Mahic et al., 2017). Maternal immune activation (MIA) is often modeled in animals to investigate the underlying mechanisms that drive this increased risk. In the MIA model, pregnant animals are administered bacterial or viral mimetics such as lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (Poly I:C), which activate a cascade of inflammatory cytokines and chemokines in the mother's bloodstream, affecting offspring development (Kentner et al., 2019; Arsenault et al., 2014; Brown et al., 2009). Administration of these mimetics is meant to model inflammatory responses, such as a fever, that a person may experience during a bacterial (LPS) or viral (Poly I:C) infection (Meyer, 2019). MIA largely affects the social (Connors et al., 2014; Zhao et al., 2021a), cognitive (Zhao et al., 2021b; Nakamura et al., 2021), sensorimotor (Inceoglu et al., 2006; Howland et al., 2012), and neurophysiological (Vojtechova et al., 2021; Zhao et al., 2021b) development of offspring. The MIA model, therefore, can be used to identify the mechanistic underpinnings of these neurodevelopmental and behavioral changes.

One hypothesis for how MIA contributes to brain and behavioral abnormalities across the lifespan is through epigenetic dysregulation and deleterious activity of transposable elements (TEs). TEs are sequences of DNA that can mobilize and insert themselves along the genome. Acting as *cis*-regulatory elements, TEs can directly affect gene transcription (Pontis et al., 2019; van de Lagemaat et al., 2003). TEs can

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be classified as Long Interspersed Nuclear Elements (LINEs), Short Interspersed Nuclear Elements (SINEs), or Endogenous Retroviruses (ERVs).

Indeed, as a major source of non-coding RNA, TEs afford organisms the ability to adapt under stressful conditions (Hunter et al., 2012, 2013, 2015; Hunter, 2020; Bartlett et al., 2021). Importantly, this adaptive mechanism can have negative effects. For example, differences in TE expression within brain regions concomitant with the hypothalamic-pituitary adrenal (HPA) axis have been associated with the etiology of numerous neuropsychiatric disorders, including autism, schizophrenia, and depression (DeRosa et al., 2022a; Lapp and Hunter, 2019; Guffanti et al., 2018).

Recent evidence has begun to tease apart the roles of the epigenome and TEs in the context of MIA across the lifespan. For example, MIA was associated with an increased expression of TEs from the class II ERVs in the fetal mouse brain 6 h post-immune challenge (Herrero et al., 2023). In addition, RNA-sequencing revealed that the most significantly downregulated gene in multiple brain regions of rhesus macaques exposed to MIA was *PIWIL2,* a key regulator of DNA methylation and retrotransposition (Page et al., 2021). MIA has also been associated with reduced expression of *Mecp2,* a protein that binds to methylated DNA to affect transcription and chromatin reorganization, in the hypothalamus of adolescent mice (Basil et al., 2014). Moreover, increased expression of LINE-1 copy number (Bundo et al., 2014) and ERVs (Herrero et al., 2023) have been observed in the prefrontal cortex of adult mice exposed to MIA. Together, these results highlight the pervasive effect of MIA on the epigenome and TEs, but it still remains unclear how this profile of pathological TE expression emerges following prenatal MIA challenge.

We have previously demonstrated an epigenetic reconfiguration of the placenta following exposure to MIA on gestational day 15 in rats (Núñez Estevez et al., 2020). *Mecp2* was significantly upregulated in the placenta of MIA exposed male and female rat offspring and increases in mRNA levels of retrotransposition repressor O-GlcNAcylation (*Ogt*) were observed in the placenta of female offspring (Núñez Estevez et al., 2020). Moreover, Herrero et al. (2023) found a differential expression of ERVs within the placenta of both sexes following MIA exposure in mice. In the present study, we hypothesized that this difference in TE and TE regulator expression following MIA challenge may be a result of global changes in heterochromatin, which is responsible for preventing deleterious TE activity and promoting overall genomic stability (Allshire and Madhani, 2018). We focused specifically on histone-3 lysine-9 trimethylation (H3K9me3) given its role in tightly regulating the activity of ERVs within the rodent placenta (Chuong et al., 2013). Moreover, in a separate experiment, we wanted to further categorize how differences in TE expression within the adult brain are associated with changes in behavior following exposure to MIA. We targeted our molecular analyses to the hypothalamus given its association with schizophrenia etiology, and its location at the top of the HPA axis, which is often perturbed in psychotic disorders and MIA models (Goldstein et al., 2007; Thompson et al., 2004). In addition, the epigenetic reconfiguration of the hypothalamus in particular appears to be one risk factor for developing behavioral abnormalities in the MIA model (Zhao et al., 2022; Núñez Estevez et al., 2020). TEs have only received a fraction of the amount of research that protein coding genes have in behavioral models (Herrero et al., 2023; Lambert et al., 2020). Understanding how TEs fit into well-established behavioral models is needed to bridge the applicability of MIA in animal models to human populations. While Herrero et al. (2023) investigated ERVs in their MIA model, given the implication of ERVs in immune dysfunction (Balestrieri et al., 2019), we wanted to expand on how MIA may affect the profile of TEs in the brain and included one of each class of retrotransposon. We specifically targeted intracisternal particle-A (IAP), LINE-1, and B2 SINE given their ability to dynamically regulate gene transcription in the brain (Bartlett et al., 2023; Gaubatz et al., 1991; Han et al., 2004). MIA model translatability, on both behavioral and physiological levels, is critical to the future identification of potential therapeutic targets (Kentner et al., 2019).

2. Methods

2.1. Animals and housing

All animal procedures were performed in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care. Protocols were approved by the Massachusetts College of Pharmacy's Institutional Animal Care and Use Committee. Male (Wilmington, MA) and female (Experiment 1: Hollister, CA; Experiment 2: Raleigh, NC) Sprague-Dawley rats were housed at 20 °C on a 12-h light/dark cycle (lights on from 0700 to 1900) in standard laboratory cages (27 × 48 × 22 cm) with food and water given *ad libitum.* Two-weeks after arriving to the facility, male and female rats were placed 1:2 in larger breeding cages (51 × 41 × 22 cm) and female estrus cycle was tracked daily. Pregnancy (Gestational day 0; G0) was determined by the presence of spermatozoa in the vaginal lavage followed by sustained diestrus. The day before drug administration (G14), females were singly housed in clean cages. For dams that carried their litters to term, the day of birth was defined as postnatal day (P)0. On P1 litters were standardized to 10 pups per litter made up of 5 males and 5 females, wherever possible. One male and one female offspring from each litter were weaned into same-sex pairs on P22. All cages contained a tube, Nestlets®, and a Nylabone® except between G18-P12, where cages only contained Nestlets in order to prevent neonatal injury.

2.2. Maternal immune activation

Experimental procedures and group assignments are outlined in Fig. 1A, Supplementary Table 1; guidelines checklist, Kentner et al. (2016), and Supplementary Table 2. **Experiment 1:** On the morning of G15 (starting at 0930), dams were weighed and intraperitonially (i.p) injected with either 200 μg/kg lipopolysaccharide (LPS; *Escherichia coli*, serotype 026:B6; L-3755, Sigma, St. Louis, MO, USA, n = 6) or an equal dose of pyrogen-free saline (n = 6). Rats were euthanized 24 h after the immune challenge using a solution of ketamine/xylazine (150 mg/kg, i.p./50 mg/kg, i.p.). Dams were then intracardially perfused with PBS. The first three conceptuses of the uterine horn were bilaterally dissected for the fetal body and placenta (Fig. 1B) as previously described (Núñez Estevez et al., 2020; Jensen Peña et al., 2012). Tissues were frozen on dry ice, then stored at −80 °C for future processing.

Experiment 2: An additional set of dams were administered either LPS (n = 10) or saline (n = 11) on the morning of G15. Dam sickness behaviors including ptosis (eyelid drooping), piloerection (ruffled fur), and lethargy were observed and each individual behavior was scored by an experimenter blind to the experimental conditions using a 3-point scale (0 = absence of behavior, 1 = mild presentation of behavior, 2 = severe presentation of behavior). Scores were taken at baseline, 30, 60, 90, 120, 240, and 360 min after receiving either injection dose and a composite score was calculated for all behaviors at each timepoint where a score of 0 = no visible signs of sickness 1–2 = mild sickness, and 3–4 = severely sick (adapted from Hayley et al., 2002; Kentner et al., 2007). This metric was used to validate the induction of an inflammatory response in the LPS treated dams (Connors et al., 2014; Kentner et al., 2016). These dams carried their pregnancies to term, and their offspring completed behavioral measures during the neonatal and adult stages of development (see below for description of behavioral measures used in Experiment 1). Offspring were weighed weekly throughout the course of the study in order to monitor general health and stress levels.

2.3. Fetal sexing (Experiment 1)

Genomic sex of each fetus was performed using polymerase chain reaction (PCR; adapted from Núñez Estevez et al., 2020; Miyajima et al., 2009). Briefly, DNA was extracted from fetal bodies using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA, Cat. # 69504) according to the manufacturer's instructions. DNA quality and

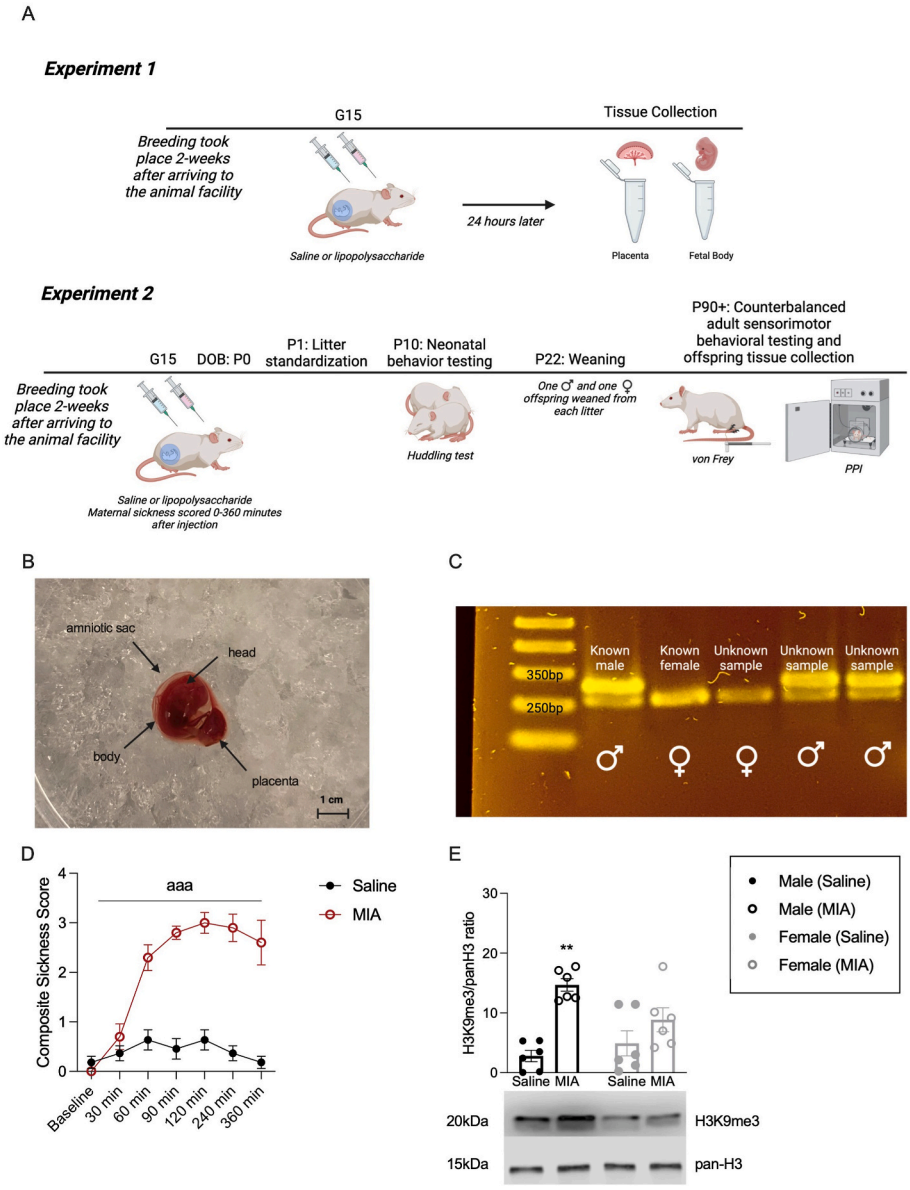

Fig. 1. Placental histone-3 lysine-9 trimethylation is significantly elevated following maternal immune activation (MIA). (A) Timeline of Experiments 1 and 2. (B) Photograph depicting the contents of each uterine conceptus. (C) Representative photograph of PCR products used to determine the sex of each sample from the fetal body. Known male and female samples were loaded prior to loading unknown samples. (D) Composite sickness behavior scores 0, 30, 60, 90, 120, 240, and 360 min after dams were injected with either saline (n = 11) or LPS (n = 10). (E) H3K9me3 expression relative to pan-H3 in placental tissues of male and female offspring 24-h after MIA exposure (Saline: n = 6; MIA: n = 6). Data are displayed as mean ± SEM. $^{aaa}p < 0.001$, main effect of MIA. $^{**}p < 0.01$, Saline versus MIA.

concentrations were determined using the Nanodrop Spectrometer 2000 (Thermoscientific). 1 μL of DNA (100 mg/mL) was mixed with PCR Supermix (Thermo Fisher Scientific, Cat. # 10572014) and primers for *Sry* and beta-actin (ACTB) were used. Primer sequences for all of the PCR analyses conducted in this study can be found in Supplementary Table 3. Each reaction was allowed to run for 35 cycles. PCR products were loaded onto a 1.8% agarose gel, DNA bands were visualized with SYBR™ Safe DNA Gel Stain (Thermo Fisher Scientific, Cat. #S33102) and gels were imaged using the Accuris™ SmartDoc™ 2.0 transilluminator system (Millipore Sigma, Cat. #Z742593). The presence of a *Sry* band (317-bp product) was used to identify males, while the presence of only a beta-actin band (240-bp product) indicated that the sample was genetically female (Fig. 1C).

2.4. Histone extractions and western blotting

Placental histone extractions were obtained from one male and one female offspring from each dam (Saline: n = 6, MIA: n = 6) 24 h after maternal immune activation according to the manufacturer's instructions (Epigentek, Cat. #OP-0006-100). Protein concentrations of each extract was determined using the BCA assay (Pierce™, Cat. #23227). Each sample was mixed with an equal amount of 2x Laemmli buffer (Bio Rad Laboratories Cat. #1610737) and denatured at 100 °C for 5 min and then cooled to room temperature. 30 μg of histones were added into each well of MiniProtean® gels (Bio Rad Laboratories, Cat. #4568101). After running the gel at 120 V for 1 hourt, proteins were transferred on nitrocellulose membranes (Bio Rad Laboratories, Cat. #1620147) and blocked in 5% nonfat milk with TBS +0.05% Tween 20 (TBST) for 1 h at room temperature. Membranes were then allowed to incubate overnight at 4 °C with the primary H3K9me3 antibody (1:1000; Abcam, Cat. #ab8898). The next day, membranes were washed for 3 × 10 min with TBSTt, and then incubated with the secondary antibody (1:10,000, Thermo Fisher Scientific, Cat # 31460) made in 1% nonfat milk for 1 h at room temperature. Membranes were washed 3 × 5 min with TBST and visualized using a chemiluminescent substrate (Thermo Fisher Scientific, Cat. #34580) for 5 min. After scanning, the membranes were stripped (Thermo Fisher, Cat. # 21062) for 15 min at 37 °C, blocked, and re-probed for 1 h at room temperature using a pan-H3 primary antibody (1:25; Abcam, Cat. #ab1791). Membranes were then washed, incubated with the secondary antibody (1:10,000, Thermo Fisher Scientific, Cat. #31460) for 1 h at room temperature, exposed to the chemiluminescent substrate, and scanned again. Densitometry was

used to determine the relative H3K9me3 to pan-H3 ratio of each sample.

2.5. Neonatal huddling (P10; Experiment 2)

Entire litters (Saline: n = 11, MIA: n = 11) completed a neonatal huddling task on P10 as described previously (DeRosa et al., 2022b; Naskar et al., 2019). For this task, all 10 pups were weighed, and labeled using a non-toxic marker to indicate the animal's sex. Pups were then placed equidistant along the perimeter of an arena (40 cm × 40 cm). The movement of the pups was video-recorded for 10-min. Using one video frame for every 30 s of the video, a researcher blind to experimental conditions calculated the total average number of pup clusters (a cluster is defined as two or more pups in physical contact). The average number of males and females engaged in a huddle was also recorded.

2.6. Mechanical allodynia and prepulse inhibition (P90+)

Adult behavioral measures took place between P92 and P93 and were counterbalanced across experimental conditions. For the von Frey test, rats (Saline: n = 11, MIA: n = 10) were placed into an acrylic cage on top of a grated floor. Mechanical allodynia was measured by raising a rigid polypropylene tip attached to a probed force transducer (Electronic von Frey Aesthesiometer, IITC, Inc, Life Science Instruments, Woodland Hills, CA, USA) to the animal's hind left paw. The tip was applied with increasing force until the animal withdrew its paw, and the threshold of applied weight (grams) indicated by the electronic meter was recorded. A total of four stimulus threshold recordings were obtained for each animal, and the average of these recordings was used as the mechanical allodynia threshold (Yan and Kentner, 2017).

For the prepulse inhibition (PPI) of the acoustic startle reflex task, rats (Saline: n = 11, MIA: n = 10) were placed into acoustic startle chambers (San Diego Instruments, San Diego, CA, USA). During habituation and throughout the testing session, 65 dB background noise was always present, even during trials without the presentation of a stimulus. For stimulus trials, rats were exposed to a 40-ms pulse of 120 dB white noise with or without the presentation of a prepulse. The intensity of the prepulse was one of three intensities: 8, 12, and 16 dB greater than the background noise. No-stimulus, pulse-alone, and prepulse-plus-pulse trials were pseudorandomly presented 10 times, and the average trial interval was 15 ± 5 s. The intensity of the startle response was recorded in millivolts (mV) by a piezoelectric accelerometer located within the animal's enclosure. %PPI for each prepulse intensity was calculated using the following formula: 1-(mean reactivity on prepulse-plus-pulse trials (mV)/mean reactivity on pulse alone trials (mV)) × 1/100 (Giovanoli et al., 2016).

2.7. Brain collection

Between P94–P96 a mixture of ketamine/xylazine (150 mg/kg, i.p./50 mg/kg, i.p.) was used to anesthetize adult offspring from experiment 1 (Saline: n = 11, MIA: n = 10). Animals were then transcardinally perfused with PBS, whole hypothalamus was dissected, frozen on dry ice, and stored at −80 °C.

2.8. PCR

Hypothalamic RNA, from the adult offspring that completed the behavioral tests, was extracted using RNeasy Lipid Tissue Kit (QIAGEN, Valencia, CA, USA, Cat. #74804) according to the manufacturer's instructions. RNA quality and concentrations were determined using the Nanodrop Spectrometer 2000 (Thermoscientific). cDNA was then generated using 1 μg of RNA and the QuantiTect Reverse Transcription Kit (QIAGEN, Valencia, CA, USA, Cat. # 205311). Qualitative real-time PCRs were carried out on cDNA samples (Saline: n = 7, MIA: n = 7) using Sybr Green Master Mix (Thermo Fisher Scientific, Cat. #A25742) and primers for the following targets: *7sk*, *Fkbp5,* IAP, B2 SINE, LINE-1 ORF-1, and LINE-1 ORF2 (Supplementary Table 3). Data were analyzed using the $2^{\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

3. Statistics

Statistical Software for the Social Sciences (SPSS) was used to perform statistical analyses. A repeated measures ANOVA was used to compare sickness behavior scores between dams that received either saline or LPS across each time point. Two-way ANOVAs (MIA x sex) were used to compare male and female relative levels of placental H3K9me3, litter weights, huddling behavior, mean %PPI, mechanical allodynia (von Frey), and relative levels of gene expression. While a two-way ANOVA did not demonstrate a main effect of MIA or sex for huddling behavior, we had the *a priori* hypothesis to test males and females separately given that the neurodevelopmental effects of the MIA model are often sex-specific (Gogos et al., 2020; Kentner et al., 2019; Zhao et al., 2021a, 2021b). A two-way repeated measures ANOVA (MIA x sex) was performed to compare differences in offspring bodyweights from weaning to adulthood in addition to group differences in %PPI across each type of trial. Animal body weights on the day of testing were used as a covariate for the von Frey test. In instances where there was a violation to the assumption of data normality (Shapiro-Wilk test), Kruskal-Wallis tests were used and are expressed as X^2. Partial eta-squared (n_p^2) is reported as a metric of effect size for the ANOVAs (0.02 = small effect, 0.13 = moderate effect, 0.26 = large effect; Miles and Shevlin, 2001). Independent-samples t-tests were used for post hoc testing because there were fewer than 3 groups. Pearson correlations were analyzed between measures of transcriptomic measures and offspring behavior. Data are visually expressed as the mean ± SEM.

4. Results

4.1. Maternal LPS administration induced a potent inflammatory response

Administration of LPS to pregnant animals elicits a cascade of inflammatory signals which can lead to the emergence of sickness behaviors such as lethargy, piloerection (rough and raised fur), and ptosis (eye drooping; Connors et al., 2014). In the present study, a repeated measures ANOVA revealed a main effect of MIA, where maternal sickness scores were significantly elevated in dams that received LPS compared to saline (Fig. 1D; Saline: n = 11, MIA: n = 10, $F(1, 19) = 85.34$, $p < 0.001$, $n_p^2 = 0.818$), thus validating the effectiveness of the LPS used in our MIA model.

4.2. MIA elevated placental H3K9me3 in a sex dependent manner

Previous work has demonstrated a sex-specific differential expression of epigenetic marks such as *Mecp2* and *Ogt* within the rat placenta following MIA (Núñez Estevez et al., 2020). To explore the possibility that this difference in gene expression may be due to a more global reconfiguration of chromatin availability within the placenta, we assessed the expression of histone packaging protein H3K9me3. A two-way (MIA x sex) ANOVA revealed a main effect of MIA ($F(1, 24) = 23.93$, $p < 0.001$, $n_p^2 = 0.545$) as well as a MIA × sex interaction ($F(1, 24) = 6.01$, $p = 0.023$, $n_p^2 = 0.231$). H3K9me3 was significantly elevated in male MIA offspring ($t(10) = -3.71$, $p = 0.004$) but not female MIA offspring ($p > 0.05$; Fig. 1E; Saline: n = 6, MIA: n = 6).

4.3. MIA affected male and female offspring sensorimotor behavior across development

We investigated the effect of MIA on offspring social thermoregulation, also referred to as 'huddling.' MIA has been shown to reduce nest seeking behavior in rat pups (Baharnoori et al., 2012), suggesting that the effects of MIA may target early sensorimotor development. After

testing males and females separately, the analysis revealed that male pups exposed to MIA formed fewer numbers of clusters compared to male pups exposed to saline (Fig. 2A and B; Saline: n = 11, MIA: n = 10, $F(1, 21) = 8.339$, $p = 0.009$, $n_p^2 = 0.782$). Huddling of female pups was unaffected by MIA ($p > 0.05$). Importantly, we did not observe significant differences in the body weight of the litters (Fig. 2C; Saline: n = 11, MIA: n = 10). Huddling efficiency has been previously associated with differences in metabolic factors such as serum triglycerides and levels of brown adipose tissue (Soriano et al., 2006; García-Torres et al., 2015). Therefore, the lack of significant differences in litter bodyweight suggests that the reduced huddling behavior in males is more likely due to an alteration in sensorimotor development, and not a difference in metabolic demand.

Rapid changes or large group differences in bodyweight are often an indicator of stress (Barnett, 1958; Barnett et al., 1960). Therefore, we monitored the bodyweights of the offspring at weaning and across the

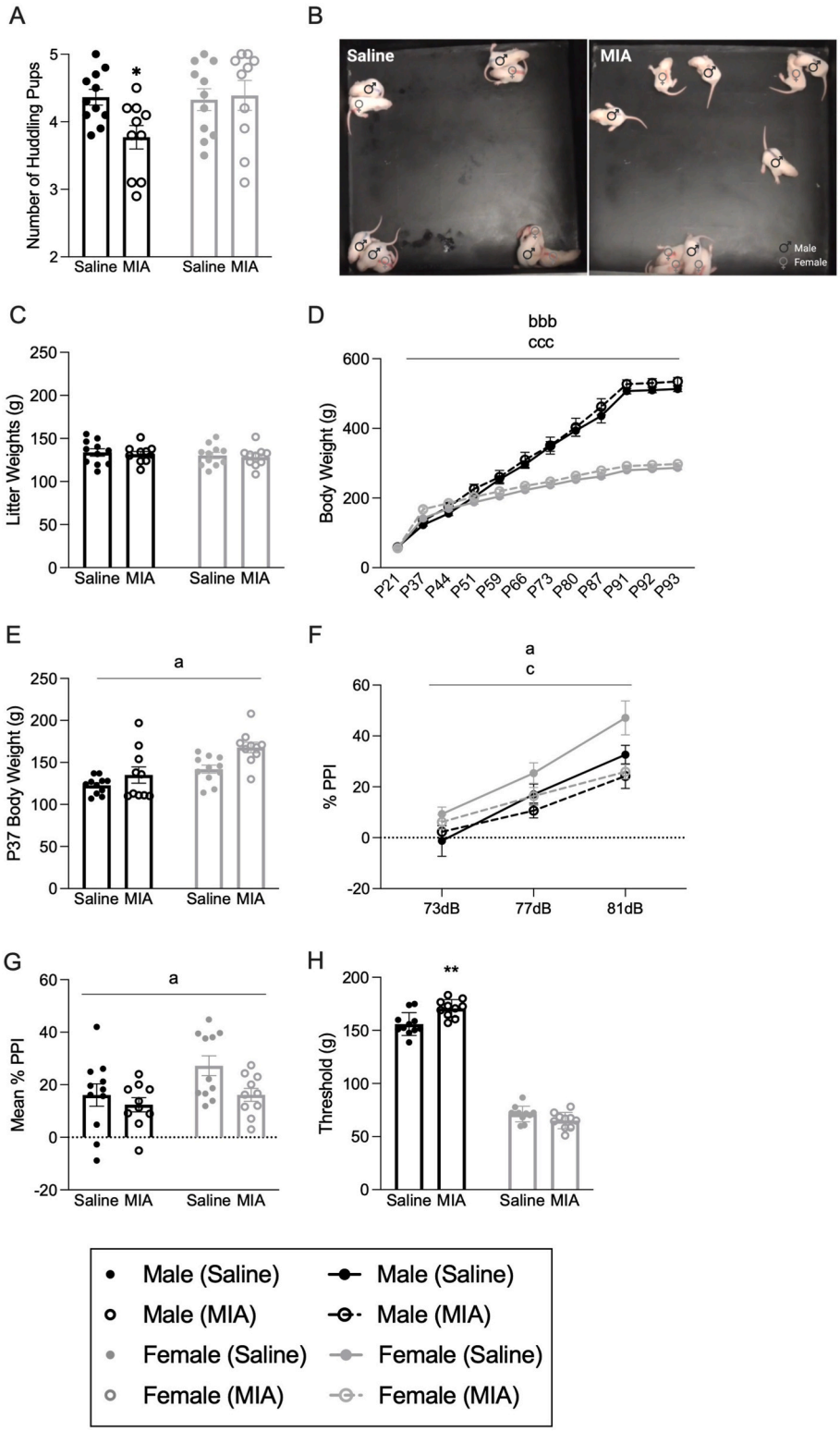

Fig. 2. Life-long physiological outcomes and sensorimotor processing in male and female offspring exposed to maternal immune activation (MIA). (A) Average number of clusters formed by male pups (black circles) and female pups (grey circles) from each litter. (B) Representative images of huddling task performance between saline and MIA-exposed litters. (C) Litter weights on postnatal day 10 (P10). Each dot represents the 5 males (black) or 5 females (grey) that were weighed together from each litter (Saline: n = 11; MIA: n = 10). (D) Bodyweights of offspring measured across the lifespan starting on postnatal day 21 (P21). (E) Bodyweights of offspring on P37. (F) Percent prepulse inhibition (%PPI) of the acoustic startle reflex across each trial type. (G) Mean %PPI collapsed across all trial types. (H) Mean mechanical allodynia thresholds in grams. Data are displayed as mean ± SEM. (Saline: n = 11; MIA: n = 10). $^{*}p < 0.05$, $^{**}p < 0.01$, Saline versus MIA. $^{a}p < 0.05$, main effect of MIA. $^{b}p < 0.05$, $^{bbb}p < 0.001$, main effect of sex. $^{ccc}p < 0.001$, main effect of time.

lifespan to ensure the physiological health of both groups. A repeated measures ANOVA only identified a main effect of sex ($F(1, 38) = 157.43$, $p < 0.001$, $\eta_p^2 = 0.806$) and time ($F(11) = 1162.5$, $p < 0.001$, $\eta_p^2 = 0.968$) on bodyweight (Fig. 2D; Saline: n = 11, MIA: n = 10). When tested individually, the only timepoint where we observed a significant effect of MIA exposure was on P37 (Fig. 2E; Saline: n = 11, MIA: n = 10, $X^2(1) = 4.53$, $p = 0.033$).

Percent inhibition of the acoustic startle reflex, or %PPI, was used to assess sensorimotor gating in adult offspring. A significant main effect of MIA revealed an attenuated %PPI response across all prepulse intensities in both males and females ($F(1, 38) = 4.6$, $p = 0.038$, $\eta_p^2 = 0.108$). There was also a significant main effect of sex, where males had reduced %PPI across all intensities compared to females ($F(1, 38) = 4.64$, $p = 0.038$, $\eta_p^2 = 0.109$) regardless of treatment group. There was no MIA × sex interaction (Fig. 2F; Saline: n = 11, MIA: n = 10). Mean %PPI was calculated by collapsing %PPI across all dB intensities. There was a main effect of MIA, where mean %PPI was significantly reduced in offspring exposed to LPS regardless of sex (Fig. 2G; Saline: n = 11, MIA: n = 10, $F(1, 41) = 5.27$, $p = 0.027$, $\eta_p^2 = 0.125$).

To further investigate these significant differences in sensorimotor processing, we tested offspring using the von Frey task of mechanical allodynia. Using bodyweight as a covariate, analyses of mechanical allodynia revealed a main effect of sex (Saline: n = 11, MIA: n = 10, $F(1, 42) = 32.2$ $p < 0.001$, $\eta_p^2 = 0.465$) and a MIA × sex interaction ($F(1, 42) = p < 0.001$, $\eta_p^2 = 0.282$). Male MIA offspring exhibited a significantly higher mechanical allodynia threshold ($t(19) = -3.51$, $p = 0.002$; Fig. 2H). Female MIA offspring did not differ from their saline counterparts ($p > 0.05$).

4.4. MIA significantly affected offspring hypothalamic transposable element expression in a sex-specific manner

Previous work has underscored the role of MIA in shaping the offspring's stress response by modulating gene expression within the hypothalamic-pituitary adrenal (HPA) axis (Maganga-Bakita et al., 2022; Connors et al., 2014). Here, hypothalamic gene expression relative to the housekeeping gene *7sk* were determined using a two-way (MIA x sex) ANOVA. There was a main effect of MIA for the expression of genes responsible for the expression and sensitivity of the glucocorticoid receptor including *Gr* (Fig. 3A; Saline: n = 7, MIA: n = 7, $F(1, 28) = 10.61$, $p = 0.003$, $\eta_p^2 = 0.307$) and *Fkbp5* (Fig. 3B; Saline: n = 7, MIA: n = 7, $F(1, 28) = 5.55$, $p = 0.027$, $\eta_p^2 = 0.188$).

We found a main effect of sex ($F(1, 28) = 8.32$, $p = 0.008$, $\eta_p^2 = 0.257$), MIA ($F(1, 28) = 12.24$, $p = 0.002$, $\eta_p^2 = 0.338$), and a MIA × sex interaction ($F(1, 28) = 19.54$, $p < 0.001$, $\eta_p^2 = 0.449$) for the relative expression of IAP, which belongs to the ERV family of retrotransposons. MIA was associated with a significant increase in IAP within the female (Saline: n = 7, MIA: n = 7, $t(6.9) = -4.56$, $p = 0.003$) but not male ($p > 0.05$) hypothalamus (Fig. 3C). In addition, there was a main effect of MIA on the expression of B2 SINE, the murine equivalent of the primate Alu retrotransposon (Fig. 3D; $X^2(1) = 4.66$, $p = 0.031$), where male MIA offspring demonstrated significantly higher levels of B2 (Saline: n = 7, MIA: n = 7, $t(6.2) = -2.7$, p = 0.030). There was also a main effect of MIA on open reading frame 1 of the LINE-1 retrotransposon (LINE-1 ORF1; Fig. 3E; Saline: n = 7, MIA: n = 7, $X^2(1) = 5.6$, $p = 0.018$). There were no significant differences in the expression of LINE-1 open reading frame 2 (Fig. 3F; $p > 0.05$).

The relationship between changes in the hypothalamic transcriptome and offspring behavior were further extrapolated using Pearson correlations. In male offspring from saline treated dams, but not MIA males, *Gr* expression was positively associated with LINE-1 ORF1 expression (r = 0.915, $p = 0.004$; Fig. 4A). In MIA exposed male offspring, B2 SINE expression positively correlated with %PPI for the 73 dB trials (r = 0.865, $p = 0.012$; Fig. 4B), while levels of IAP negatively correlated with %PPI for the 77 dB trials (r = −0.823, $p = 0.023$; Fig. 4C). In MIA female offspring, B2 SINE expression positively

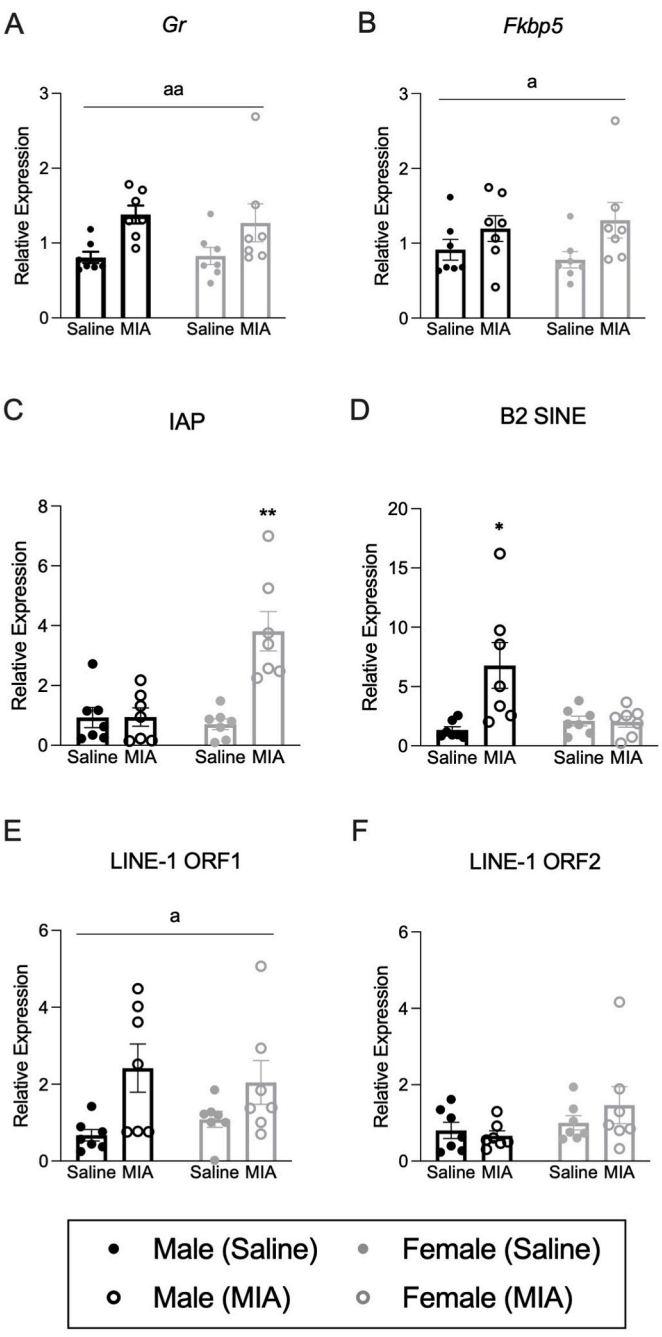

Fig. 3. Maternal immune activation (MIA) is associated with changes in the genetic and non-genetic profile of the hypothalamus. Relative expression levels of (A) *Gr*, (B) *Fkbp5*, (C) IAP, (D) B2 SINE, (E) LINE-1 ORF1, and (F) LINE-1 ORF2 to *7sk* within the hypothalamus of adult offspring. (Saline: n = 7; MIA: n = 7). Data are displayed as mean ± SEM. $^*p < 0.05$, $^{**}p < 0.01$, Saline versus MIA. $^ap < 0.05$, $^{aa}p < 0.01$ main effect of MIA.

associated with total mean %PPI (r = 0.771, $p = 0.042$; Fig. 4D). A summary of all findings can be found in Supplementary Table 4.

5. Discussion

Our data demonstrate the pervasive effect of MIA on offspring development and behavior. Specifically, MIA was associated with an increase in H3K9me3 in the placenta of male fetuses. MIA outcomes often differ by sex, as does the pathogenesis of MIA-related disorders such as schizophrenia (Gogos et al., 2020; Kentner et al., 2019; Bale

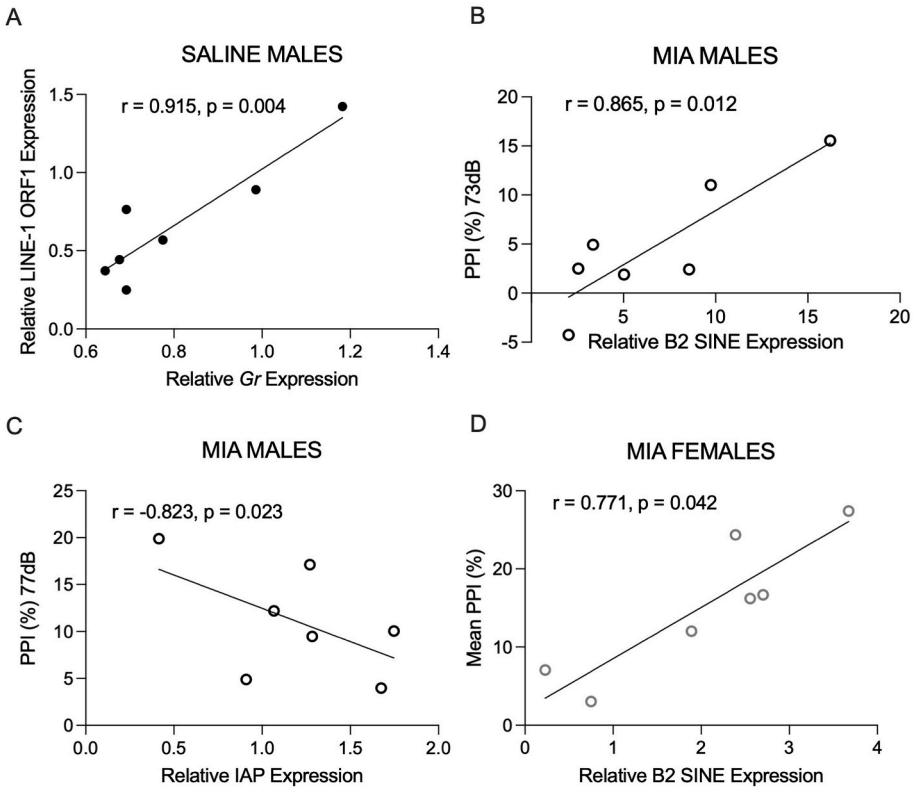

Fig. 4. Pearson correlations between hypothalamic gene, transposable element (TE) expression, and the behavior of male and female offspring gestationally treated with either saline or LPS. Pearson correlations for (A) relative *Gr* and LINE-1 ORF1 expression in saline males, (B) relative B2 SINE expression and %PPI for 73 dB trials in MIA males, (C) relative IAP expression and %PPI for 77 db trials in MIA males, and (D) relative B2 SINE expression and total mean %PPI in MIA females. Data are expressed as $p < 0.05$.

et al., 2010; Goldstein et al., 2007). Indeed, we have previously shown a female-specific increase in a different placental TE repressor, *Ogt* (Núñez Estevez et al., 2020). This effect was also observed in the placenta of female mouse fetuses prenatally exposed to chronic stress (Nugent et al., 2018), and *Ogt* was shown to regulate levels of placental H3K27me3 within this group (Nugent et al., 2018). Collectively, this work supports the idea that separate mechanisms related to the chromatin landscape may be associated with the developmental and behavioral outcomes in male and female offspring following early life stress. However, the role of H3K27me3 within the MIA model specifically needs to be explored further. A schematic summarizing the results of the present study are outlined in Fig. 5. While these data provide a critical first step in implicating H3K9me3 in the MIA model, one major limitation to this study is that it's still unknown if changes in placental H3K9me3 are also associated with changes in TE expression within the placenta or even fetal brain. The present study supports the hypothesis that a disruption in the "typical" chromatin landscape of the placenta is a risk factor for future neurodevelopmental pathology, but the immediate downstream effects of this disruption require a deeper analysis. It's also unclear where H3K9me3 is localizing. In our previous acute stress models H3K9me3 has tended to localize to TEs within the brain (Hunter et al., 2012; Bartlett et al., 2021). Future work should consider performing chromatin immune precipitation sequencing (ChIP Seq) of placental or fetal brain samples. Such experiments are a critical next step in identifying where in the DNA these histone modifications, or even other epigenetic mechanisms, are mobilizing to. While our data provide an important first step in establishing H3K9me3 in the male-specific response to MIA, much more work is needed to understand the full functional implication of this global change in the heterochromatin landscape.

In the behavioral experiments, neonatal huddling was reduced in MIA male offspring, which is in-line with previous research of nest seeking behavior (Baharnoori et al., 2012). We showed no significant differences between MIA and saline litter bodyweights, suggestive of sensorimotor-specific deficits rather than an artifact in metabolic demand since huddling is a form of thermoregulation. In adulthood, MIA was associated with decreased sensorimotor gating in both sexes, and this has been previously reported in other MIA models (Basta-Kaim et al., 2011; Wolff and Bilkey, 2010) as well as in studies of individuals with schizophrenia (Swerdlow et al., 2014; Kumari et al., 2007). In the von Frey test, we observed an increased mechanical allodynia threshold in adult male MIA offspring. While lower thresholds have been reported in P30 male MIA poly (I:C) exposed mice (Zhao et al., 2022), measures of mechanical allodynia can differ across developmental ages (Zouikr et al., 2015) or based on the type of immunogen used to induce MIA (Arsenault et al., 2014). Like PPI, our findings translate to what has been observed in clinical populations, as individuals with schizophrenia can experience an insensitivity to painful stimuli (Stubbs et al., 2015). Together, these behavior results demonstrate general deficits in sensorimotor processing following prenatal exposure to gestational infection.

The hypothalamus is largely sensitive to early life perturbations and has been implicated in the sex-specific pathogenesis of disorders like schizophrenia (Bale et al., 2010; Goldstein et al., 2007). Specifically, disruptions of hypothalamic function within the stress axis have been linked to neuropsychiatric disorder etiology (Pariante, 2009; Mikulska et al., 2021). In the context of MIA, the differential expression of genes and epigenetic marks within the hypothalamus has been associated with a range of behavior abnormalities including those related to sensorimotor processing (Zhao et al., 2022; Núñez Estevez et al., 2020). Here, we show an upregulation of *Fkbp5* and *Gr* in adult offspring of both sexes that were exposed to MIA. Early life stress has been associated with an increase in hypothalamic *Fkbp5* (Ke et al., 2018), which serves as a co-chaperone for *Gr* to inhibit HPA-axis feedback (Zannas and Binder, 2014). Elevated levels of *Fkbp5* are therefore associated with *Gr* resistance, which may help to explain the increase in *Gr* we observed here.

Psychiatric disorders are often associated with changes in the expression and regulation of several TEs (DeRosa et al., 2022; Lapp and Hunter, 2019), suggestive of a compounding effect of TEs. During embryonic development, LINE-1 is essential for the progression from the 2-cell stage to blastocyst (Percharde et al., 2018). The functional

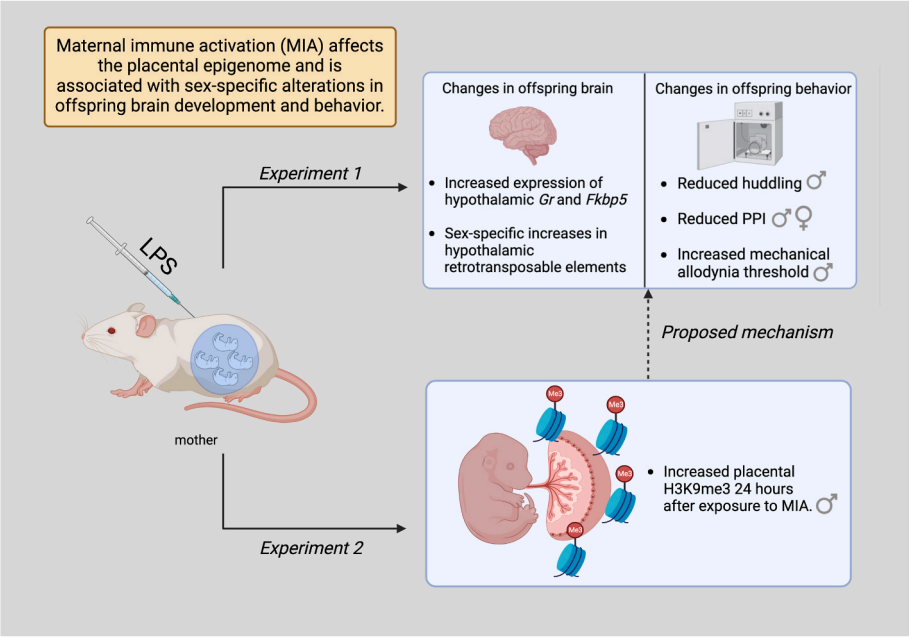

Fig. 5. Maternal immune activation (MIA) is associated with an elevation of histone-3 lysine-9 trimethylation (H3K9me3) in the placenta. This global change in heterochromatin availability may be one mechanism by which MIA drives the future aberrant expression of transposable elements and associated deficits in sensorimotor processing in male and female rat offspring. LPS: *lipopolysaccharide.* Me3: symbol of histone trimethylation used in this figure to represent H3K9me3.

relevance of differences in TE expression within the adult brain, however, is still being unpacked. We demonstrate a positive association between *Gr* and LINE-1 ORF1 expression within the hypothalamus of control male offspring, but not MIA offspring. LINE-1 ORF1 expression can differ by both sex and brain region (Cuarenta et al., 2021). Our data support a relationship between *Gr* and LINE-1 in male adult rats, and future studies localizing LINE-1 ORF1 to *Gr* will be able to offer more information surrounding how these two interact to potentially influence each other's expression. Within the male MIA offspring, we observed a significantly negative correlation between %PPI and the relative expression of IAP and B2 SINE. These results supported our hypothesis that deleterious TE activity in the hypothalamus is related to behavior abnormalities in the MIA model. Interestingly, we observed the opposite effect in females exposed to MIA, as B2 SINE expression positively correlated with %PPI. As a non-coding RNA, B2 SINE is known to interact directly with RNA polymerase II to block transcription. Unlike IAP, however, B2 SINE has been shown to be elevated in the male rat hippocampus, and this elevation was associated with more active coping strategies (Lambert et al., 2020). Our data further support a likely role of B2 SINE in the emergence of sex differences in behavior.

Although we did not observe significant correlations between measures of mechanical allodynia and TEs, it's plausible to assert that our results may better be explained by the compounding effect of multiple TEs instead of the differential expression of one individual TE. It's also possible that mechanical allodynia may be more closely linked with the activity of TEs not included in the present analyses. Importantly, these results collectively demonstrate a relationship between MIA-associated behavioral alterations and differences in TE expression. The functional role of TEs in behavioral paradigms still needs to be parsed out. Future studies may consider incorporating agents such as histone deacetylase (HDAC) or histone acetyltransferase (HAT) inhibitors to artificially manipulate TE activities during behavioral tasks. Moreover, future studies should consider that the rats used in our gene analyses completed the full battery of behavior tasks. Although animal handling and tasks were standardized across groups, behavior task experience and experimenter handling can affect gene expression (Meaney and Aitken, 1985; 1988).

Increased TE expression throughout the brain has been linked to an array of neuropsychiatric conditions (DeRosa et al., 2022a; Lapp and Hunter, 2019; Guffanti et al., 2018). Yet, research assessing TEs and their association with behavioral phenotypes in animal models of psychiatric disorders is still in its infancy. Recently, deficits in %PPI were shown to positively correlate with the degree of ERV expression within the medial prefrontal cortex of MIA mice (Herrero et al., 2023). Here, in addition to their decreased PPI performance, female MIA offspring displayed an increase in IAP expression in the hypothalamus. MIA was also associated with higher levels of B2 SINE and LINE-1 ORF1 in both sexes. The specific functional contributions of this differential TE expression need to be studied further. Nonetheless, these results offer an exciting step in further defining the role of TEs in contributing to behavioral abnormalities associated with early life stress.

6. Conclusions

Data from the present study demonstrates the pervasive effects of MIA on neurodevelopment and behavior. We found an increase in stress-related genes and TEs within the hypothalamus in tandem with sensorimotor processing deficits as revealed by the PPI and von Frey tasks. In a separate study, placental tissue from MIA-exposed male offspring exhibited an increase in global H3K9me3 24 h post-challenge. While these data provide a first step in implicating chromatin architecture in the placenta as part of a potential mechanism that drives MIA-associated effects, there is much more work that needs to be done to test this hypothesis directly. Nevertheless, our results provide one example by which prenatal insults disrupt the genomic stability of the maternal fetal interface. Future studies, especially those using animal models of neuropsychiatric disease, should integrate assessments of TEs and epigenetic activities.

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CRediT authorship contribution statement

Holly DeRosa: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Arianna Smith:** Investigation, Writing – review & editing. **Laurel Geist:** Investigation, Writing – review & editing. **Ada Cheng:** Investigation, Writing – review & editing. **Richard G. Hunter:** Conceptualization, Supervision, Resources, Writing – review & editing. **Amanda C. Kentner:** Supervision, Conceptualization, Formal analysis, Funding acquisition, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2023.100538.

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