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Maternal immune activation epigenetically regulates hippocampal serotonin transporter levels

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ABSTRACT

Major depressive disorder (MDD) is one of the most debilitating psychiatric diseases, affecting a large percentage of the population worldwide. Currently, the underlying pathomechanisms remain incompletely understood, hampering the development of critically needed alternative therapeutic strategies, which further largely depends on the availability of suitable model systems.

Here we used a mouse model of early life stress – a precipitating factor for the development of MDD – featuring infectious stress through maternal immune activation (MIA) by polyinosinic:polycytidilic acid (Poly(I:C)) to examine epigenetic modulations as potential molecular correlates of the alterations in brain structure, function and behavior. We found that in adult female MIA offspring anhedonic behavior was associated with modulations of the global histone acetylation profile in the hippocampus. Morevoer, specific changes at the promoter and in the expression of the serotonin transporter (SERT), critically involved in the etiology of MDD and pharmacological antidepressant treatment were detected. Furthermore, an accompanying reduction in hippocampal levels of histone deacetylase (HDAC) 1 was observed in MIA as compared to control offspring.

Based on these results we propose a model in which the long-lasting impact of MIA on depression-like behavior and associated molecular and cellular aberrations in the offspring is brought about by the modulation of epigenetic processes and consequent enduring changes in gene expression. These data provide additional insights into the principles underlying the impact of early infectious stress on the development of MDD and may contribute to the development of new targets for antidepressant therapy.

1. Introduction

Major depressive disorder (MDD) is a highly prevalent psychiatric disorder affecting an estimated 10–15% of the population worldwide (Bromet et al., 2011). It is characterized by the persistent presence of several emotional, psychological and somatic symptoms including, but not limited to depressed mood, anhedonia, fatigue, sleep disturbances, cognitive dysfunctions and suicidal ideation (American Psychiatric, 2013). In most developed and high-income countries, MDD has thus consistently been ranked among the top five public health issues with regard to socioeconomic burden (Murray et al., 2012).

Akin to other neuropsychiatric illnesses, the currently available therapeutic interventions for MDD exhibit several unfavorable factors, limiting treatment success. Among these are the poorly understood latency in the onset of the clinical effect of most commonly used antidepressant medications, a high incidence of adverse side effects and the considerable amount of non-responders among the affected MDD patients (Holtzheimer and Mayberg, 2011; Whiskey and Taylor, 2013). Furthermore, the heterogeneity and complexity of the disorder suggest a complex set of interactions between several endogenous and exogenous variables contributing to the pathogenesis of MDD, the underlying mechanisms of which still remain poorly understood. Elucidation of the

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pathophysiological principles at the molecular level, however, constitutes a prerequisite for the development of alternative therapeutic strategies and is largely dependent on the availability of specific animal models. Considering the relevance of chronic stress as a precipitating factor for the development of MDD (Bagot et al., 2014; Slavich and Irwin, 2014), several stress-based animal models of depression exist, featuring paradigms of stress exposure at various stages of life, including the prenatal period (Hammels et al., 2015 Markham and Koenig, 2011; Meyer, 2014). Animal models of prenatal infectious stress have recently been emerging as powerful tools allowing to test the role of early immune stimulation as a priming factor that induces long-lasting neuronal changes contributing to the emergence of depression later in life (Reisinger et al., 2015). One frequently used rodent model of maternal immune activation (MIA) is based upon the systemic administration of polyinosinic:polycytidilic acid (Poly(I:C)), a synthetic analog of double-stranded RNA (dsRNA) and activator of toll-like receptor (TLR) 3 (Tatematsu et al., 2014), to the pregnant dam in order to mimic a gestational viral infection (Meyer, 2014 Reisinger et al., 2015; Smith et al., 2007). Using this model, we have recently demonstrated behavioral, neural and molecular alterations associated with depression in the adult male offspring (Khan et al., 2014). Here we employed the Poly(I:C) MIA model to test the hypothesis that the long-lasting nature of the impact of prenatal infectious stress on brain structure and function, at the molecular, cellular and behavioral levels, is associated with a modulation of epigenetic mechanisms, central mediators of the impact of environmental influences on brain and behavior (Bagot et al., 2014; Tsankova et al., 2007). In addition to examining the consequence of MIA on key elements of epigenetic regulation globally in the hippocampus, a pivotal brain region in the neural circuitry of depression (McKinnon et al., 2009; Posener et al., 2003), we specifically investigated its effect on the serotonin transporter (SERT) - critically involved in the etiology of MDD and pharmacological antidepressant treatment .

2. Materials and methods

2.1. Animals

All mice used were C57BL/6N mice obtained from Charles River (Sulzfeld, Germany). Behavioral and molecular experiments were carried out in separate cohorts of adult female offspring (8–12 weeks) from maternal immune activation at embryonic day 12.5 (PIC) and age matched control animals. A diagram depicting the time course and experimental design of this study is provided in Fig. 1.

Animals were housed under standard conditions, all animal procedures were approved by the national ethical committee on animal care and use in Austria (Bundesministerium für Wissenschaft und Forschung: BMWF-66.009/0015-II/3b/2012) and were carried out according to international laws and policies. All efforts were made to minimize animal suffering and discomfort and to reduce the number of animals used.

2.2. Timed mating and maternal immune activation

Timed mating procedures were carried out following an established protocol, as previously described (Khan et al., 2014). Upon confirmation of a vaginal plug in the morning following the mating period, this time was denoted as embryonic day 0.5 (E0.5) (Simard et al., 2010).

On E12.5 the total weight gain (%) since mating was calculated for each dam. C57BL/6 mice can be expected to gain 25%–40% of their initial body weight during the first 12.5 days of pregnancy, allowing the determination of pregnancy with up to 99% certainty at this time point (Hau and Skovgaard Jensen, 1987). Pregnant mice were subjected to either MIA or control treatment at E12.5, since the effect of Poly(I:C)-induced MIA on depression-related behavior and associated neurobiological changes in mice has been previously described at this time point (Khan et al., 2014).

For MIA, pregnant mice were injected intraperitoneally (i.p.) with 20 mg/kg Poly(I:C) (Polyinosinic–polycytidylic acid potassium salt, Sigma Aldrich, St. Louis, MO, USA) dissolved in vehicle or the vehicle alone (physiological saline solution, 0.9%, Fresenius Kabi, Bad Homburg, Germany). The injection volume for both conditions was 10 ml/kg.

2.3. Behavioral testing

All behavioral experiments were carried out during the light phase of the light-dark cycle.

2.3.1. Sucrose preference test (SPT)

The experimental protocol used has been previously described in detail (Savalli et al., 2015). Briefly, mice were food and water restricted for 18 h prior to a two-bottle forced choice test (regular drinking water *vs* 2% sucrose (Sigma Aldrich, St. Louis, MO, USA) solution) conducted over a 3 h testing period. The consumption of sucrose solution relative to the total liquid consumption was evaluated and the percentage of sucrose preference calculated from this measure.

2.3.2. Forced swim test (FST)

The forced swim test was carried out as previously described (Khan et al., 2014; Monje et al., 2011). Briefly, mice were placed into glass beakers filled with tap water (~22 °C) for 6 min. Their mobility was recorded and automatically analyzed using the software Videotrack (Viewpoint, Champagne au Mont d'Or, France) for the evaluation of the proportion of time spent immobile during the last 4 min of the test.

2.3.3. Elevated plus maze (EPM)

For the EPM test, mice were placed in the center of a plus-shaped maze (elevated ~50 cm off the ground) consisting of two opposing open arms and two opposing closed arms, the latter of which were enclosed by ~ 20 cm high walls. The movement within the maze was tracked using Videotrack (Viewpoint, Champagne au Mont d'Or, France), and the proportion of time spent in the open arms during the 5 min test period was calculated, as described elsewhere (Walf and Frye, 2007).

2.3.4. Open field test (OFT)

For the OFT, the locomotor activity of mice in a testing arena (27.3 cm × 27.3 cm) equipped with infrared beam arrays (MedAssociates Inc., St. Albans, VT, USA) was evaluated for 60 min. Data were analyzed using the software Activity Monitor (MedAssociates Inc., St. Albans, VT, USA) to calculate the total distance travelled, as previously described (Khan et al., 2014).

2.3.5. Rotarod

In the Rotarod test the motor coordination of mice was evaluated using an automated system consisting of a rotating drum and the accompanying software (MedAssociates Inc., St. Albans, VT, USA). During a 6-min test period the rotation speed was increased steadily from 4 rpm to 40 rpm and the latency of the animal to fall off the drum was recorded and averaged over three trials for each mouse, as previously described (Khan et al., 2014).

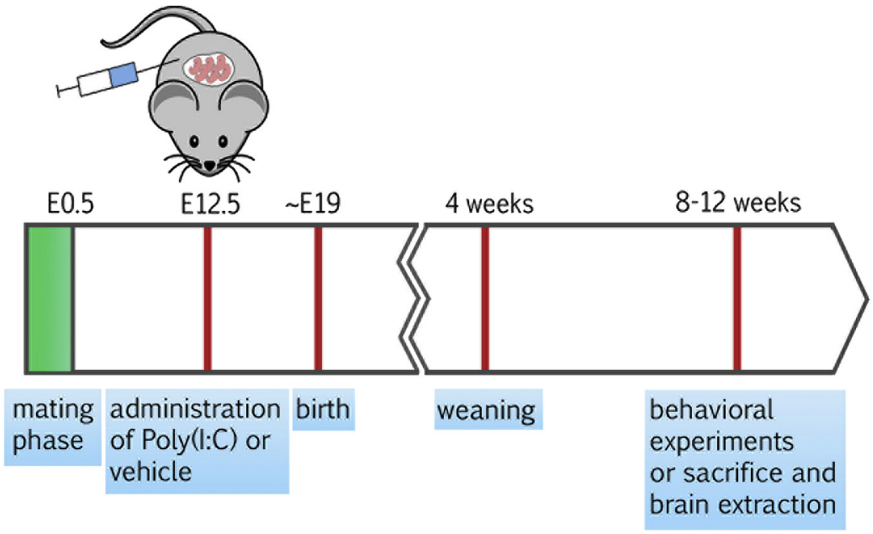

Fig. 1. Timeline of experimental paradigm for MIA using Poly(I:C). Time points of events and procedures are shown: Mice were mated for 12 h; pregnant females were injected with Poly(I:C) at E12.5; pups were born at approx. E19 and weaned at 4 weeks old; behavioral experiments or sacrifice and brain extraction were performed at the age of 8–12 weeks in separate cohorts.

2.4. Brain tissue extraction

All mice were sacrificed by cervical dislocation. Brains were quickly extracted and hippocampi were bilaterally dissected from each animal.

Western Blot experiments, mRNA analysis and chromatin immunoprecipitation (ChIP) were carried out using tissue from parallel cohorts of mice.

For ChIP and Western Blot experiments, the tissue was placed in reaction tubes, flash-frozen using liquid nitrogen immediately following dissection, and then stored at −80 °C until used for analysis. mRNA samples were stored in RNAse-free reaction tubes containing RNAlater (Ambion, Austin, TX, USA) and stored at −20 °C for subsequent experimental work-up.

2.5. Molecular biology and biochemistry experiments

2.5.1. Western Blot analysis

Standard Western Blot analysis (Monje et al., 2011) was performed on hippocampal tissue of PIC and control mice. Briefly, tissue was homogenized in 1 M PBS followed by lyzation in a protein lysis buffer (10 mM Tris−HCl pH 7.5, 150 mM NaCl, 1% SDS, 0.5% Triton X-100, 1 mM EDTA, 10 mM NaF, 5 mM $Na_4O_2P_7$, 10 mM Na_3VO_4 and 1× protease inhibitor cocktail (ThermoScientific, Waltham, MA, USA)).

Protein concentration was determined using the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's instructions and using a Synergy Multi-Mode Microplate Reader (Biotek, Winooski, VT, USA) for spectroscopic measurements. 50 μg of total protein was mixed with 5 μl Loading Buffer (ThermoScientific, Waltham, MA, USA) and subjected to SDS-PAGE electrophoresis followed by blotting onto a PVDF membrane. Membranes were incubated with primary antibodies (SERT: 1:1000, Santa Cruz, Dallas, TX, USA; β-actin: 1:2000, US Biological, Salem, MA, USA; H3: 1:1000, Abcam, Cambridge, UK; acH3: 1:1000, Millipore; H4: 1:1000, Millipore; acH4: 1:1000, Millipore, Billerica, MA, USA) overnight at 4 °C followed by a one-hour incubation at room temperature (RT) with the appropriate secondary antibody (SERT: Donkey anti-goat IgG-HRP, 1:3000, Santa Cruz, Dallas, TX, USA; β-actin: Goat anti-mouse IgG-HRP, 1:3000, Cell Signaling Technology; all histones: anti-rabbit IgG-HRP, 1:3000, Cell Signaling Technology, Danvers, MA, USA). Pierce ECL Western Blot Substrate (Pierce Biotechnology, Rockford, IL, USA) was used to develop the membranes according to manufacturer's instructions. Quantification was performed by chemiluminescent imaging with a FluorChem HD2 (Alpha Innotec, Kasendorf, Germany) using the respective software. Values obtained from densitometry of target proteins (SERT, acetylated H3 and H4) were normalized (β-actin for SERT, total H3 and H4 for acetylated H3 and H4) for the semi-quantitative determination of protein levels as described elsewhere (Griesauer et al., 2014).

2.5.2. RNA isolation, cDNA synthesis and qRT-PCR

mRNA was extracted using the RNEasy Mini Kit (Qiagen, Venlo, Netherlands) according to the protocol supplied by the manufacturer. 900 ng of mRNA was used for the subsequent cDNA synthesis using the DyNAmo cDNA Synthesis Kit (ThermoScientific, Waltham, MA, USA) following the manufacturer's instructions.

cDNA samples (dilutions: SERT and HDAC4 1:1, HDAC1 and HDAC5 1:5, β-actin 1:10) were used for qRT-PCR assessment of the relative levels of hippocampal mRNA and HDACs. For each sample repeat, 7.5 μl of SYBR Green MasterMix (LifeTechnologies, Carlsbad, CA, USA), 0.15 μl each of forward (sequences HDAC1: CCGCATGACTCATAATTTGCTG; HDAC4: GGCCCACCGGAATCTGAAC; HDAC5: TCTTGTCGAAGTCAAAGGAGG, Invitrogen, Waltham, MA, USA) and reverse (sequences HDAC1: TGTGAGGGCGATAGATTTCCAT; HDAC4: GCTGCGTTTTCCCGTACCA; HDAC5: GAGGGGAACTCTGGTCCAAAG, Invitrogen, Waltham, MA, USA) primers, 6.2 μl of RNase-free water and 1 μl of sample were added to a well of a RT-PCR plate. All reactions were carried out in duplicates. C(t) values were obtained for each sample as well as a concomitant determination of the housekeeping gene β-actin (sequences forward: ATGGTGGGAATGGGTCAGAAG; reverse: TCTCCATGTCGTCCCAGTTG, Invitrogen, Waltham, MA, USA).

The C(t) values for β-actin were used for calculation of ΔC(t), representing the relative quantification of mRNA amounts in each sample. This further allowed the calculation of ΔΔC(t), subtracting the mean ΔC(t) value of the control group from the mean ΔC(t) value for the Poly(I:C) group. ΔΔC(t) was then used to express the fold change of mRNA levels observed between Poly(I:C)-exposed mice and untreated mice, using the formula $2^{-\Delta\Delta C(t)}$.

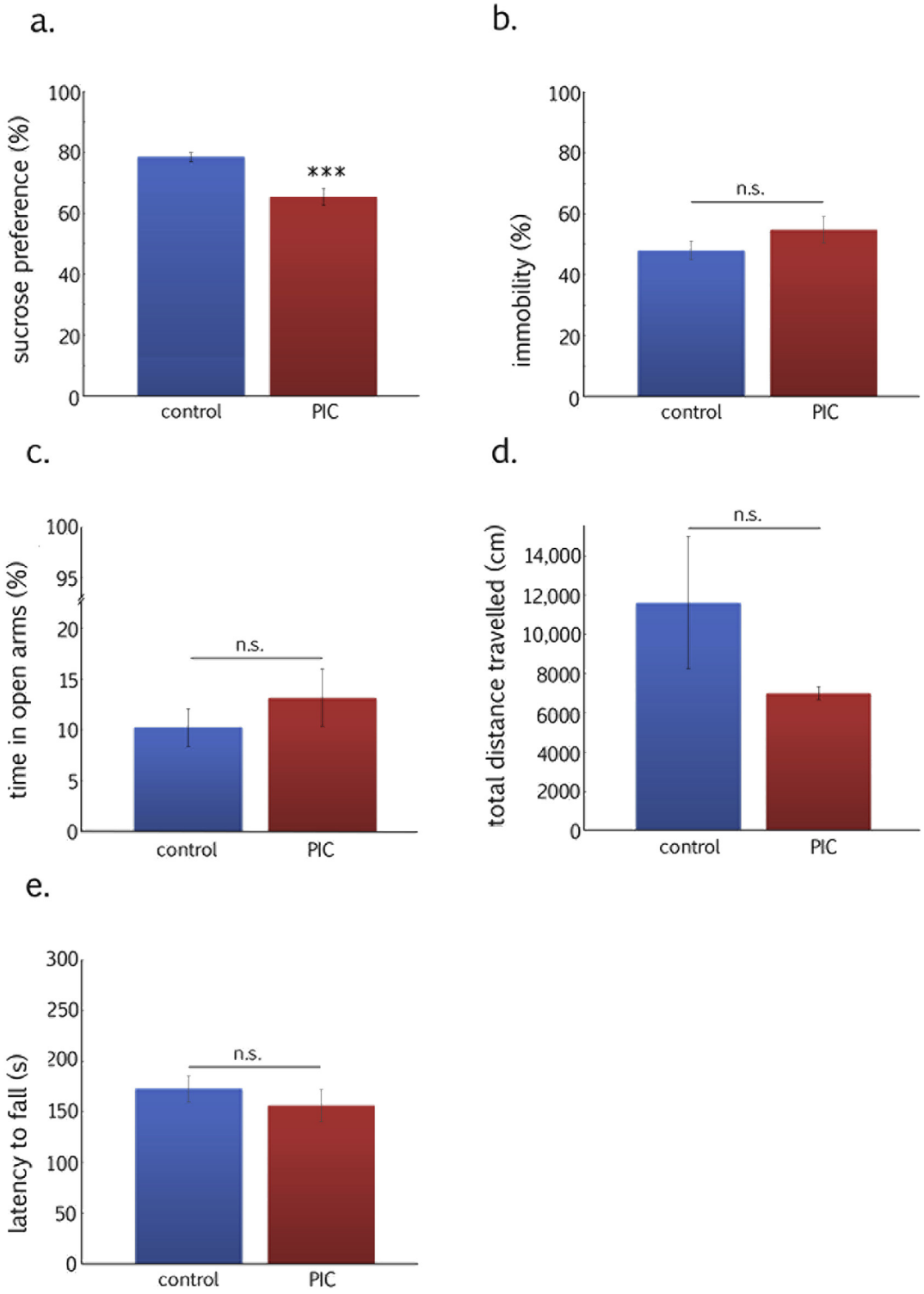

Fig. 2. MIA increases anhedonic behavior in adult female offspring (PIC), but does not affect behavioral despair, anxiety-related behavior or general behavior. **a**. Analysis of anhedonic behavior of control vs. PIC mice in the sucrose preference test [control n = 37; PIC n = 15]. **b**. Freezing behavior of control vs. PIC mice during the forced swim test [control n = 26; PIC n = 14]. **c**. Analysis of anxiety-related behavior of control vs. PIC mice in the elevated plus maze [control n = 26; PIC n = 14]. **d**. Total locomotor activity of control vs. PIC mice in the open field test [control n = 26; PIC n = 14]. **e**. Analysis of motor coordination of control vs. PIC mice in the Rotarod test [control n = 26; PIC n = 14]. Data are presented as mean ± SEM; ***: $p \leq 0.001$; n.s. not significant.

2.5.3. Chromatin immunoprecipitation

For Chromatin immunoprecipitation (ChIP) analysis of H3 and H4 acetylation at the SERT promoter, hippocampal tissue of two mice of the same treatment group (randomly selected across different litters to eliminate any possible litter effects) and sex were pooled to obtain one ChIP sample. The protocol employed was adapted from Nelson et al. (2006).

Briefly, hippocampal tissue was homogenized in 1.42%

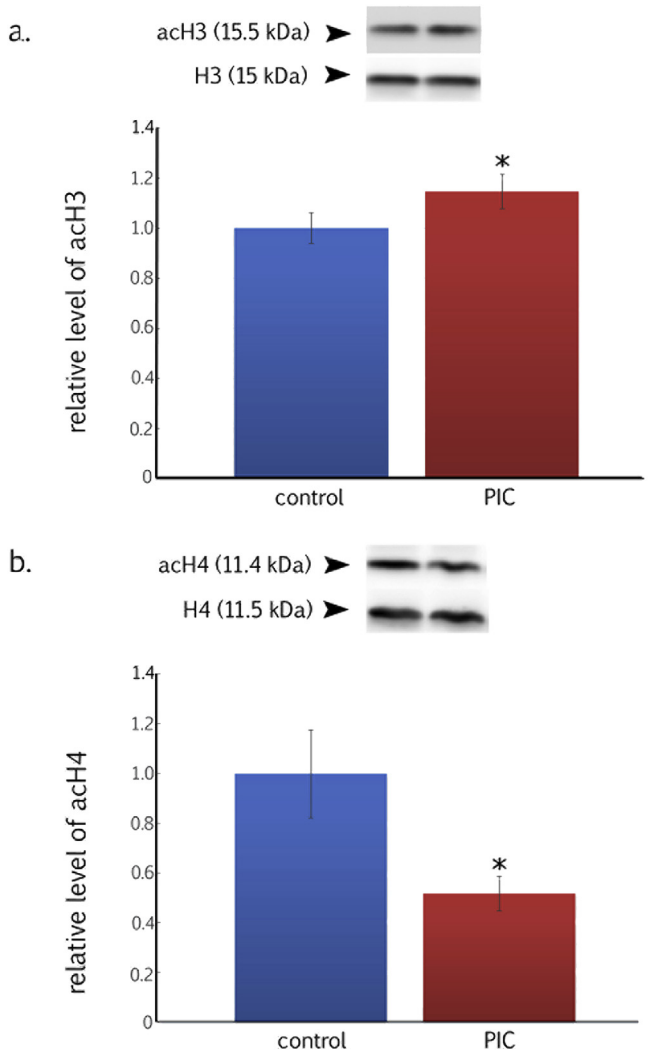

Fig. 3. MIA alters global levels of histone acetylation in the PIC hippocampus. **a**. Result of Western blot analyzing protein levels of acH3 vs. total H3 in the hippocampus of control vs. PIC mice [control n = 5; PIC n = 5]. **b**. Result of Western blot analyzing protein levels of acH4 vs. total H4 in the hippocampus of control vs. PIC mice [control n = 5; PIC n = 5]. Data are presented as mean ± SEM (relative to controls); *: $p \leq 0.05$.

formaldehyde in PBS (1 M) using a hand homogenizer, followed by cross-linking, quenching (125 mM glycine), washing (PBS) and lysis in low salt IP buffer with protease inhibitors (IP Buffer, low salt: 150 mM NaCl, 50 mM Tris–HCl/pH 7.5, 5 mM EDTA, 0.5% NP-40, 1% Triton X-100; Halt protease inhibitors added just prior to use in 1:100 dilution, ThermoScientific, Waltham, MA, USA). After cell lysis, samples were centrifuged (2000 G, 4 °C, 5 min), washed (low salt IP buffer), centrifuged again and resuspended in 500 μl low salt IP buffer. Shearing of chromatin was accomplished using a sonicator bath (Bioruptor Plus, Diagenode, Liege, Belgium) after which samples were equally divided into three parts for immunoprecipitation with antibodies (anti-acetyl-H3, Millipore; and anti-acetyl-H4, Millipore, Billerica, MA, USA) and mock immunoprecipitation (without antibodies). 7 μl of antibody (anti-acH3 and anti-acH4) or IP buffer (for mock reaction) and 20 μl of protein A magnetic beads (Magna ChIP Protein A Magnetic Beads, Millipore, Billerica, MA, USA) were added and samples were incubated overnight at 4 °C on a rotating platform. The following day, samples were washed and a magnetic rack (Magna GrIP Rack, Millipore, Billerica, MA, USA) was utilized to pellet the samples. The final pellet was resuspended in 100 μl PBS (1 M) and 1 μl of proteinase K (20 ug/ul) and incubated for 30 min on a heated shaker (55 °C) After high speed centrifugation, samples were boiled at 95 °C for 15 min to dissociate the magnetic beads from the antibodies. The magnetic beads were removed using the magnetic rack and the PCR-ready DNA samples were transferred to new reaction tubes and stored at - 20 °C until used for qRT-PCR.

For qRT-PCR, master mixes containing SYBR Green Master Mix (12.5 μl per sample repeat, LifeTechnologies, Carlsbad, CA, USA) and SERT primers (4 μl each, sequences forward: CAGAGCTCTCAGTCTTGTCTCC; reverse: TGCTGGTCAGTCAGTGGTG; Invitrogen, Waltham, MA, USA) were prepared and 4.5 μl of each sample was added per reaction. All samples were analyzed in duplicates. C(t) values for each sample and IP condition were recorded and used for statistical analysis. Briefly, qRT-PCR data were analyzed as described above, except that instead of β-actin C(t) values, mock IP C(t) values were used as internal controls, accounting for potential differences in input tissue amounts and non-specific binding of the magnetic beads in each IP condition.

2.6. Statistical analysis

For comparisons between two groups (PIC versus control mice) in the behavioral and all molecular and biochemical experiments, two-sample t-tests (two-tailed, equal variance) were carried out and p-values ≤ 0.05 were considered statistically significant. All calculations were conducted using Microsoft Excel.

3. Results

3.1. Adult female PIC offspring display enhanced depression-related anhedonia but show no alterations in behavioral despair, anxiety-related and general behavioral functions

SPT analysis was performed in order to characterize the effect of MIA on depression-related anhedonia in female PIC offspring as compared to control mice. A statistically significantly reduced sucrose preference ($p \leq 0.001$, Fig. 2a) was observed in PIC females.

No significant difference between PIC and control mice was observed in behavioral despair as reflected in the time spent immobile during the FST (Fig. 2b). Similarly, mice from the PIC and control groups spent comparable proportions of time in the open arms of the EPM (Fig. 2c). Locomotor activity in the OFT, evaluated by the total distance travelled (Fig. 2d), and motor coordination, as measured by the latency to fall off the Rota rod (Fig. 2e), was not significantly different between PIC and control mice.

3.2. Acetylation of histone H3 and H4 is altered in the hippocampus of adult female PIC offspring

In light of the relevance of epigenetic mechanisms for long-lasting alterations in neuronal structure and function and associated mental illnesses (Tsankova et al., 2007), we next set out to determine functionally relevant histone modifications in the hippocampus of PIC and control mice. Western Blot analyses demonstrated that the global relative level of acetylated histone H3 was significantly increased in hippocampal tissue of PIC mice ($p \leq 0.05$, Fig. 3a). The ratio of acetylated H4 versus total H4 was decreased in the hippocampus of PIC mice ($p \leq 0.05$, Fig. 3b).

3.3. Hippocampal HDAC1 levels are reduced in adult female PIC offspring

Seeking to examine the molecular mediators of altered histone acetylation patterns, levels of histone deacetylating enzymes

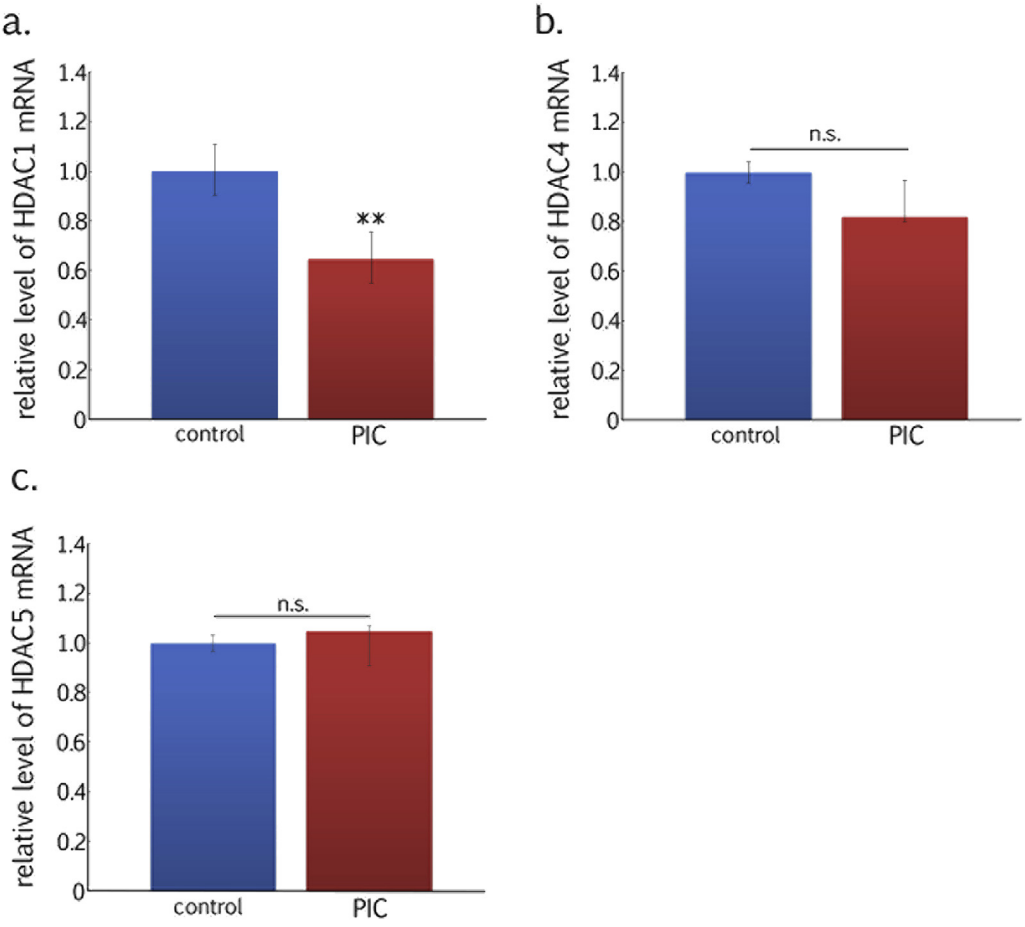

Fig. 4. MIA alters hippocampal mRNA levels of select HDACs in PIC mice. **a**. Relative mRNA level of HDAC1 in control vs. PIC mice [control n = 6; PIC n = 8]. **b**. Relative mRNA level of HDAC4 in control vs. PIC mice [control n = 6; PIC n = 7]. **c**. Relative mRNA level of HDAC4 in control vs. PIC mice [control n = 7; PIC n = 7]. Data are presented as mean ± SEM (relative to controls); **: $p \leq 0.01$; n.s.: not significant.

(HDACs) were examined by qRT-PCR in the hippocampus of PIC and control mice. Preliminary experiments (data not shown) showed that among HDACs 1-11, only HDACs 1, 4 and 5 were expressed at detectable levels. Hence, these HDACs were further analyzed for potential differences between PIC and control mice. A significant reduction in relative levels of HDAC1 mRNA was observed in PIC animals ($p \leq 0.05$, Fig. 4a), while no differences for HDAC4 and HDAC5 expression were found between groups (Fig. 4b, c).

3.4. Histone H3 and H4 acetylation is enhanced at the SERT promoter in PIC offspring

In order to investigate whether global alterations in histone acetylation profile were also detectable at the promoter sites of specific genes, Chromatin Immunoprecipitation (ChIP) analysis was employed, focusing on SERT, given its pivotal role in the pathophysiology of depression and therapeutic action of pharmacological antidepressant drugs (Haase and Brown, 2014). A significant increase in acetylated H3 and H4 associated with the SERT promoter in the hippocampus of PIC mice was revealed ($p \leq 0.05$, Fig. 5a and b).

3.5. PIC offspring display altered hippocampal SERT expression

Aiming to ascertain whether the observed modifications of histone structures at the SERT promoter translated into alterations in protein expression, hippocampal SERT levels were determined in PIC and control mice Indeed, Western Blots analysis revealed about 50% increase in hippocampal SERT protein in PIC as compared to control mice ($p \leq 0.01$, Fig. 5c).

4. Discussion

The presented results suggest that maternal immune activation may exert its effect on offspring behavior via the long-term modulation of epigenetic mechanisms, which in turn can lead to alterations in gene expression, in particular of SERT.

We first confirmed the effect of developmental infectious stress exposure through MIA by the viral mimetic Poly(I:C) at E12.5 on depression-like behavior in adult female offspring. As previously described for male progeny (Khan et al., 2014), we also observed enhanced anhedonic behavior in female offspring after MIA, manifesting in significantly reduced sucrose preference in the SPT. In contrast, the FST showed that - as opposed to male PIC offspring - behavioral despair was not increased in adult female PIC offspring. Other behavioral tests, including the OFT, EPM and Rotarod, showed no differences in the behavior of female PIC vs. control offspring, in line with our previous report on males (Khan et al., 2014).

While these data further support the general relevance of MIA in mice as a model for specific symptoms reflecting depressive-like behavior, they also suggest a potential applicability of the paradigm in the investigation of the biological basis of gender differences in depression. In humans, depression has been repeatedly reported to affect women almost twice as often as men (Angst et al.,

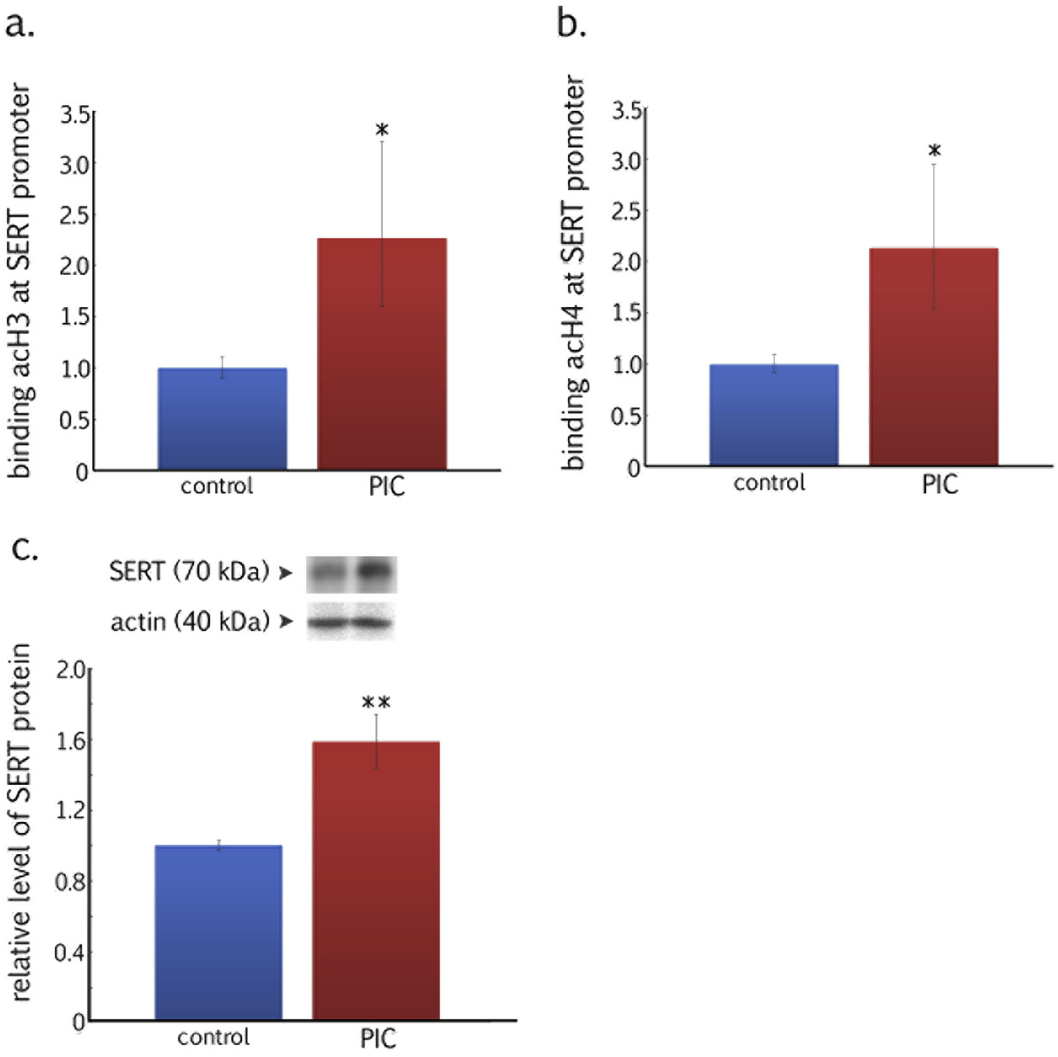

Fig. 5. MIA alters levels of histone acetylation at the hippocampal SERT promoter and increases hippocampal SERT protein in PIC mice. **a**. Binding of acH3 at SERT promoter in the hippocampus of control vs. PIC mice [control n = 6; PIC n = 6]. **b**. Binding of acH4 at SERT promoter in the hippocampus of control vs. PIC mice [control n = 6; PIC n = 6]. **c**. Results of Western blot analyzing hippocampal levels of SERT vs. actin protein in control vs PIC mice [control n = 5; PIC n = 7]. Data are presented as mean ± SEM (relative to controls); **: $p \leq 0.01$; *: $p \leq 0.05$.

2002 Bebbington et al., 1998; Kuehner, 2003 Silverstein, 1999; Weissman and Klerman, 1977). Additionally, gender differences in symptoms associated with depressive episodes have been reported, with appetite disturbances highlighted as one major difference between women and men (Marcus et al., 2005 Romans et al., 2007; Wenzel et al., 2005). It is plausible to speculate that altered experience of reward, which relates to anhedonia, may underlie these gender specificities in people and are mimicked by the herein observed sex differences in response to prenatal infectious stress in mice. Indeed, the enhancement in anhedonic behavior in female PIC offspring is slightly larger than the one found in males (Khan et al., 2014). By contrast, a higher sensitivity of male rodents to the development of behavioral despair in the FST has been described in different animal models of depression (Alonso et al., 1991; Gómez et al., 2014) and is thus in line with the present findings in the PIC paradigm. Overall, these first insights into the sex-differences in behavioral responses to gestational infection highlight the need for further investigations into their neurobiological underpinnings and translational implications.

In an attempt to understand how gestational infection may lead to long-term alterations in brain structure and function at the molecular, cellular and behavioral level, selected epigenetic markers in the adult offspring brain were examined for changes induced by MIA treatment. Epigenetic regulation of gene expression (i.e. genetic control, independent of DNA sequence) is widely thought to represent a molecular substrate for the interaction between genes and the environment leading to long-lasting alterations in transcription and persistent physiological and pathophysiological functional consequences. Indeed, numerous studies propose a critical role for aberrant transcriptional regulation in the pathophysiology of several psychiatric disorders including MDD (Charney et al., 2004; Krishnan and Nestler, 2008). Recently, first reports on the effects of prenatal infectious stress induced by MIA on selected epigenetic markers have been emerging (Basil et al., 2014 Connor et al., 2012; Tang et al., 2013). In contrast to previous studies, we here focused on the global histone acetylation profile in the adult offspring hippocampus, a central brain structure involved in the neural circuitry of MDD (McKinnon et al., 2009; Posener et al., 2003).

In particular, acetylation of H3 and H4 histones was deemed a suitable candidate mechanism, since it has been previously implicated in the pathophysiology of MDD, as well as being proposed as

a molecular effector mediating long-lasting *Gene* × *Environment* interactions (Liu et al., 2008; Tammen et al., 2013).

To date, most studies investigating histone acetylation in association with a depression-related phenotype have used stress paradigms during the early postnatal phase or in adulthood and have mostly reported decreased H3 acetylation, with one study showing an increase in H4 acetylation (reviewed in Bagot et al. (2014)). Thus while these studies support a general relevance for H3 and H4 acetylation in the pathophysiology of depression, the particular circumstances specifically inducing H3 and/or H4 modifications remain to be delineated as well as their respective contributions to the etiology of depression.

With respect to MIA, there has only been one other study investigating levels of histone acetylation: Tang et al. (2013) reported no significant effect of MIA on levels of histone acetylation in the hippocampus in adult mice, although the group noted a trend for reduced H4 acetylation, partially lending support to results of the present study (Tang et al., 2013). However, the currently available information does not yet allow for the establishment of causal associations between the observed alterations in global

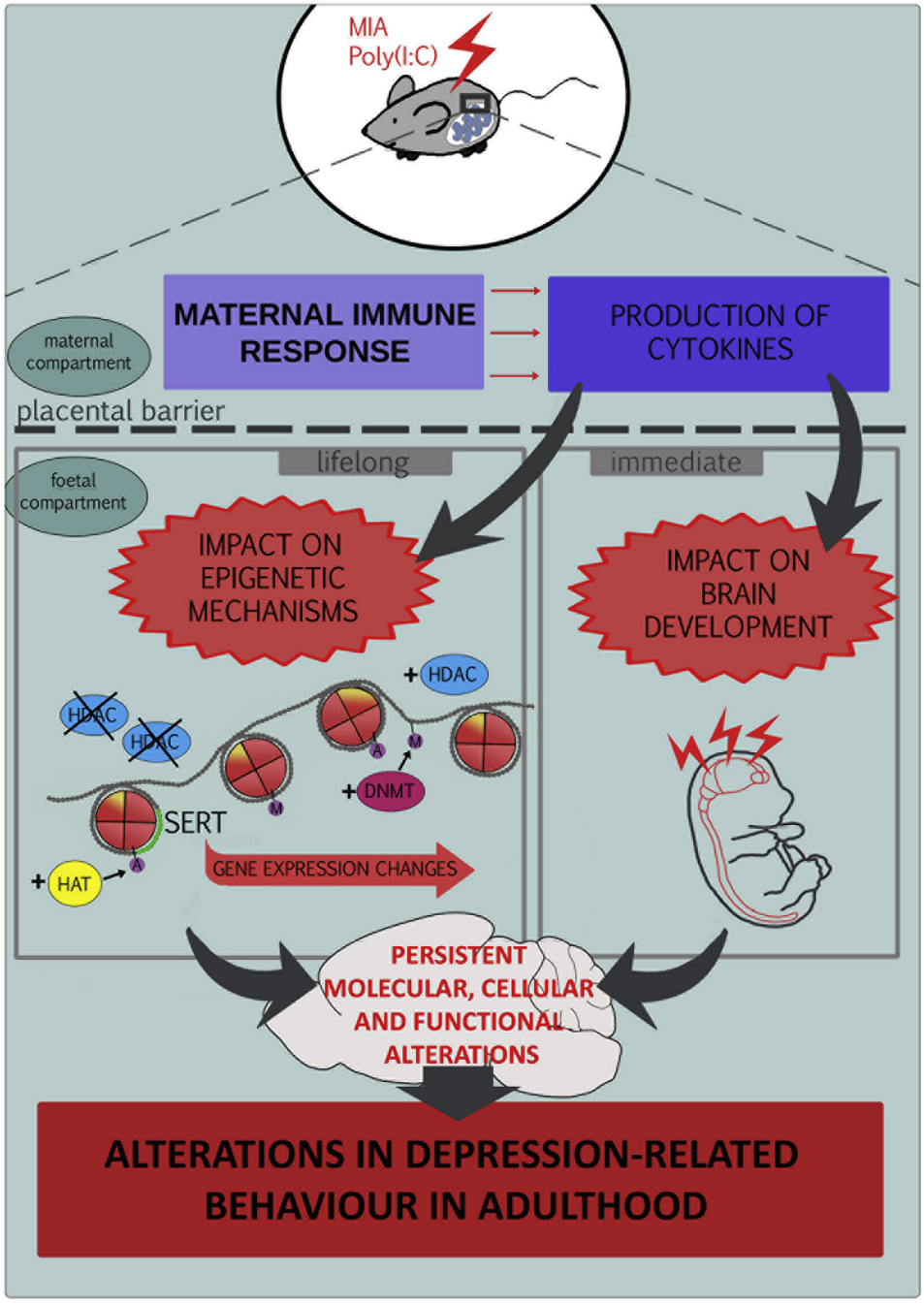

Fig. 6. Proposed model of the long-term impact of MIA on depression-related behavior and associated molecular, cellular and functional alterations in the offspring brain. Upon MIA, the maternal immune reaction includes the activation of cytokines which can readily cross the placental barrier into the fetal compartment. Through yet uncharacterized signal transduction pathways, these cytokines are proposed to influence epigenetic mechanisms in the long term, leading to changes in gene expression. Cytokines, together with this aberrant gene expression, may also directly interfere with correct brain development. Consequently, important alterations at the molecular, cellular and functional levels occur which lead to long-lasting behavioral modifications in the offspring. Histone deacteylase (HDAC); Histone acetyl transferase (HAT); DNA methyltransferase (DNMT).

hippocampal H3 and H4 acetylation and the behavioral disturbances resulting from MIA.

Rather, they suggest the dynamic regulation of H3 and H4 acetylation in the PIC hippocampus that may serve as a foundation for future in-depth investigations into the involved regulatory mechanisms.

In order to gain first insights into the intracellular mechanism potentially mediating the observed changes in histone acetylation in PIC mice, we examined expression of several HDACs, which – besides histone acetyl-transferases (HATs) - are considered the major regulators of histone acetylation (Peserico and Simone, 2011). While most HDACs (HDAC2, 3, 6, 7, 8, 9, 10, 11) were not sufficiently expressed in our samples to allow pertinent analysis of their levels, the selective reduction in HDAC1 expression observed in the PIC hippocampus proposes this enzyme as a specific mediator of the impact of MIA on epigenetic regulation in the offspring brain. Interestingly, while its defined role remains to be elucidated, evidence for an involvement of HDAC1 in the pathophysiology of MDD and in the effects of pharmacological antidepressant treatment has been emerging lately from studies using various animal models (Covington et al., 2011 Schmauss, 2015; Schroeder et al., 2013).

It is conceivable that the modulation of HDAC1 levels in the PIC hippocampus may contribute to the observed increase in global H3 acetylation as well as the specific effects at the SERT promoter. However, it cannot explain the decrease in H4 acetylation. In this respect, it is important to consider that HDAC activity need not necessarily relate to expressional levels. However, due to the lack of commercially available specific HDAC1 activity assays one cannot yet directly relate changes in HDAC expression to activity, far less to the observed changes in histone acetylation and behavior in PIC mice. Furthermore, HATs may play just as important a role in the regulation of histone acetylation levels and thereby gene expression – but remain even less well studied than HDACs in the context of depression and have not yet been investigated within the MIA paradigm at all.

We next set out to explore potential gene-specific epigenetic regulations, focusing on SERT, a key molecule involved in MDD-related serotonergic signaling and prominent target of antidepressant drugs. The more than two-fold increased binding of both acH3 and acH4 histones to the SERT promoter in the PIC hippocampus indicates SERT as specific target of the MIA-induced regulation of the epigenetic machinery which may underlie or contribute to the depression-like phenotype observed in MIA offspring. At the molecular level, hippocampal SERT protein expression was significantly increased in the MIA-exposed group which is in agreement with the fact that acetylation of histones has previously been linked to enhanced gene transcription (Shahbazian and Grunstein, 2007; Turner, 2014). However, while the focus of this study lay on the relevance of histone acetylation, other epigenetic mechanisms, such as DNA methylation, linked to MDD in different contexts (Domschke et al., 2014), may also act to regulate SERT expression. The observed increase in SERT levels in PIC mice, which display depression-like behavior, is in agreement with the generally held view that a reduction in SERT function (such as during administration of an SSRI) produces an antidepressant effect (Haase and Brown, 2014).

5. Conclusions

The present study provides evidence for alterations in several epigenetic processes in the adult female offspring brain resulting from MIA and proposes altered hippocampal SERT expression as one specific molecular effector. Furthermore, to the best of our knowledge, this is the first demonstration of an effect of MIA on histone modifications specifically at the SERT promoter. While acknowledging that other mechanisms may be involved, we propose that MIA-dependent regulation of adult hippocampal SERT expression is – at least in part – mediated by changes in histone (H3 and H4) acetylation at the SERT promoter. As such, this study supports the general notion that epigenetic mechanisms contribute to the environmental programming of brain development and behavior by embedding the impact of early life experience on gene expression (Fig. 6). Moreover, the specific effect on SERT, together with the already documented involvement of SERT in MDD, suggest that SERT holds a critical role in the development of depression-related traits associated with early life infectious stress. In conjunction with previous findings investigating the influence of other exogenous adverse events at different stages of life, our data further highlight the complexity of *Gene* × *Environment* interactions involved in the development of MDD – of which MIA constitutes one specific contributing factor.

Future analyses may build upon the data presented herein and may yield valuable information about the temporal course of the effect of MIA on SERT function and dependent behaviors as well as the precise molecular pathways employed. Jointly, this information may then allow the identification of critical windows suitable for the application of preventive interventions and potentially point towards new therapeutic targets for the treatment of depression.

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