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Title: Enhanced inflammatory status and altered metabolism in adolescence as early biological changes predictive the long-term negative consequences of exposure to stress early in life: novel targets for prevention.

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ABSTRACT

Early life stress, especially when experienced during the prenatal period or childhood, affects the brain developmental trajectories leading to an enhanced vulnerability for stress-related psychiatric disorders later in life. Although both clinical and preclinical studies clearly support this association, the biological pathways deregulated by such exposure, and the effects of early life stress in shaping the neurodevelopmental trajectories, have so far been poorly investigated.

By using the prenatal stress (PNS) model, a well-established rat model of early life stress, we performed transcriptomic analyses in the prefrontal cortex of rats exposed or not to PNS and sacrificed at different postnatal days (PNDs 21, 40, 62). We first investigated the mechanisms and pathways affected by exposures to PNS that may contribute to the long-lasting vulnerability of developing altered behaviours in adulthood (at PND62). Moreover, by focusing on transcriptomic changes, we evaluated the effects of PNS in shaping brain trajectories with the aim to identify the most critical temporal window of vulnerability, when biological alterations are already present, but clear symptoms not manifested yet.

In adult rats (PNDs 62), PNS modulates 389 genes which resulted to be involved mainly in the stress and inflammatory system response. Moreover, when we looked at temporal trajectories in term of gene expression, we found the most significant effects of PNS during adolescence (between PND40 versus 21) with an effect on pathways related to stress, inflammation and metabolism that was then maintained until adulthood.

Our data suggest that molecules belonging to the stress, inflammatory and metabolic systems may serve as biomarkers of risk to allow the identification of adolescents that have been exposed to adversities and are at high risk to develop mental illness later in life, and that thus could benefit from early preventive interventions with novel pharmacological or non pharmacological interventions able to target these biological systems.

Key words: early life stress; childhood trauma; transcriptomic profile; inflammation; metabolism; vulnerability

INTRODUCTION

It is now well known that exposures to early life stress are associated with long-lasting consequences on several biological systems that render the body and the brain more vulnerable to develop both physical and mental disorders (Debost et al., 2017; Plant et al., 2016). Indeed, exposure to adversities early in life has been associated with an increased risk of developing a large spectrum of psychiatric disorders, such as depression or psychosis later in life (De Bellis et al., 2019; Mayo et al., 2017; Tursich et al., 2014; Zeugmann et al., 2013), but also physical diseases, such as diabetes (Huffhines et al., 2016), cardiovascular disorders (Lei et al., 2018), obesity (Palmisano et al., 2016) and cancer (Holman et al., 2016).

Early life stress includes not only the classic exposure to childhood traumatic events but also adverse experiences that occur during the prenatal period, when the foetus is exposed directly to adverse events in utero, or later in life during critical neurodevelopmental phases, especially in adolescence. It is well recognized that events *in utero* have long-term influences on the disease risk. This phenomenon, known as '*early life programming'*, has been extensively studied in relation to maternal depression and stress in the mother, whereby the developing foetus is exposed to an adverse intra-uterine environment, leading to abnormal programming of its brain and the body (Goldstein et al., 2016; Lindsay et al., 2019; Reynolds et al., 2013; Van den Bergh et al., 2017), which in turn it is responsible of an enhanced vulnerability to develop a wide spectrum of abnormalities including altered behaviours (Lahti et al., 2017; Simons et al., 2019). For example, it has been shown that offspring from mothers suffering from depression during pregnancy showed suboptimal neurobehavioral functions in several clusters measured using the Neonatal Behavioural Assessment Scale clusters; interestingly, these behavioural features correlated with infant stress response and with an increased risk for developmental delay and internalizing and externalizing problems later in childhood (Osborne et al., 2018; Toffol et al., 2019).

Also, childhood represents a vulnerable temporal window for the development of altered behaviours, as the brain is still under maturation, and adverse events occurring in these periods shift the normal brain developmental trajectories versus an abnormal one, that in turn shape the brain for the development of a vulnerable phenotype later in life. Several studies have indeed shown that traumatic events in childhood are

highly correlated with an increased risk for depression, with an onset of early vulnerable traits already during adolescence (Jaworska-Andryszewska and Rybakowski, 2019). In particular, emotional abuse has been associated with the highest risk of depression, followed by neglect, sexual abuse, domestic violence and physical abuse (Mandelli et al., 2015). Paul and collaborators reported that emotional neglect was the strongest predictor for depression in boys, whereas emotional abuse was in girls (Paul and Eckenrode, 2015). Clinical findings are supported by preclinical data, where many paradigms of stress early in life, such as the prenatal stress or maternal separation, induce long lasting behavioural alterations, such as anxiety and depressive -like behaviour (Giovanoli et al., 2013; Grigoryan and Segal, 2013; Laloux et al., 2012; Luoni et al., 2017) and also cognitive deficits (Bengoetxea et al., 2017; Cattaneo et al., 2019; Guan et al., 2016).

Although the clinical and pre-clinical evidences clearly support the association between stress early in life and the enhanced risk of the exposed child to develop altered behaviours, a major gap concerns the knowledge on the biological pathways and mechanisms that are altered by such adversities. There are however some candidate systems that have been examines, such as the Hypothalamic-Pituitary-Adrenal (HPA) axis and the immune and inflammatory systems. For example, several studies showed that individuals that have been exposed to childhood trauma have a hyperactivity of the HPA axis, denoted by increased plasma adrenocorticotropin (ACTH) and cortisol levels in response to dexamethasone/corticotropin-releasing factor administration (Heim et al., 2008). Similarly, Lu and colleagues in 2016 reported higher morning cortisol awakening levels in depressed patients with childhood trauma as compared with non-exposed patients. Interestingly, higher morning cortisol awakening levels have been observed also in adult individuals with a history of abuse even without having developed a psychopathology (Lu et al., 2016), suggesting that childhood trauma per se causes alterations in the functionality of the HPA axis. Moreover, higher cortisol levels during the day are observed also in children with traumatic experiences (Pfeffer et al., 2007). Animal models of stress early in life show the presence of altered corticosterone levels both in basal condition (Brunton and Russell, 2010) as well as after a stressful challenge (Szuran et al., 2000), supporting the presence of an altered functionality of the HPA axis.

Another candidate biological process that has been associated with exposure to stress early in life is the inflammatory system. Danese and collaborators were among the first to demonstrate in 2007 that maltreated children have a more pronounced pro-inflammatory profile in term of elevated plasma C-reactive protein levels as well as of other inflammatory factors as compared with non-maltreated children (Danese et al., 2007). These findings have been then confirmed by other subsequent studies, summarized in a recent meta-analysis which supports the association between the exposure to different types of childhood trauma and the presence of a pro-inflammatory state, measures as high C-reactive protein (CRP), IL-6 and TNF-α (Baumeister et al., 2016). Inflammation has been also suggested to mediate the effect of childhood trauma on depression development, as more pronounced levels of inflammatory cytokines have been found in depressed patients with early life stress as compared with non-exposed depressed patients (Grosse et al., 2016; Muller et al., 2019; Peters et al., 2019). Moreover, Müller and colleagues have reported a significant positive association, in depressed patients, between plasma IL-6 levels and sexual abuse and physical neglect (Grosse et al., 2016; Muller et al., 2019; Peters et al., 2019). Interestingly, childhood trauma is a strong predictor of non-response to antidepressant drugs, and higher levels of inflammation can be detected in non-responder patients and represents a strong predictor, when measured at baseline, of the future response to treatment (Cattaneo et al., 2018; Cattaneo et al., 2013). Finally, preclinical data also support the association between early life stress and alterations in the inflammatory status, not only at peripheral levels, but also in the brain. For example, rats exposed to prenatal stress (PNS) show increased IL-1β and TNF-α in hippocampus and an increased activation of microglia compared with control animals, suggesting that PNS induces a basal pro-inflammatory status in stressed rats (Diz-Chaves et al., 2013; Diz-Chaves et al., 2012). Moreover, the administration of Lipopolisaccaride (LPS) to animals previously exposed to PNS induced a more pronounced induction of the pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α, suggesting that PNS caused per se an activation of the central immune system which is also hyper-reactive to a subsequent pro-inflammatory stimulus (Diz-Chaves et al., 2012).

Based on this to this background, in this paper we aimed at identifying the mechanisms and pathways which are affected in the brain (prefrontal cortex, PFC) of animals exposed to stress early in life (PNS) and that may contribute to the long-lasting vulnerability to develop altered behaviours in adulthood. Moreover, we

wanted to evaluate the neurodevelopmental trajectories affected by PNS, in order to identify the most critical temporal window of vulnerability, where biological alterations are already manifested even in the absence of clear behavioural alterations. To reach these aims, we performed a whole genome transcriptomic analyses in the prefrontal cortex (PFC) of adult animals (postnatal day, PND 62) exposed or not to PNS. Moreover, to identify the effect of PNS on brain developmental trajectories, we conducted the transcriptomic analyses both in controls and PNS animals at earlier time-points indicative of adolescence and early adulthood (PND 21 and PND 40). Identifying such mechanisms of risk trajectories could be translated into clinical biomarkers for the identification of adolescents at the highest risk and in new targets for the development of novel pharmacological interventions.

MATERIALS AND METHODS

Prenatal Stress model

Prenatal Stress (PNS) procedure was performed as already published (Cattaneo et al., 2018; Luoni et al., 2016). Briefly, pregnant dams, during the last week of gestation, were restrained in a transparent Plexiglas cylinder, under bright light, for 45 min, three times a day for 1 week. Control pregnant females were left undisturbed in their home cages. Male offspring from control and PNS groups were sacrificed at different postnatal days (PNDs), PND 21, 40 and 62 and different brain regions including the prefrontal cortex (PFC) were dissected and immediately frozen. Rat handling and experimental procedures were performed in accordance with the EC guidelines (EC Council Directive 86/609 1987) and with the Italian legislation on animal experimentation (D.L. 116/92), in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. RNA samples from the PFC of animals exposed or not to PNS at different PNDs (n=10 per group) were used for the Whole Genome transcriptomic analyses.

RNA isolation and microarray analyses

Total RNA was isolated according to the manufacturer's protocols from the rat brains using PureZol RNA isolation reagents (Bio-Rad Laboratories, Hercules, CA, USA). RNA quantity and quality were assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). All the RNA samples have been also analysed for their integrity by

using the Agilent Bioanalyzer (Agilent) and we obtained RNA Integrity Number (RIN) >8 for all the samples.

Whole Genome expression microarray analyses

Transcriptomic analyses were performed in PFC of rats, sacrificed at different PNDs (PND 21, 40, 62), using the Rat Gene 2.1st Array Strips, which covers 27,147 coding transcripts. Briefly, 250 ng of total RNA were used to synthesize second strand cDNA with the GeneChip® WT PLUS Reagent Kit (Affymetrix, Santa Clara, CA, USA). Subsequently, purified cDNA was fragmented, and 5.5 µg of fragmented cDNA were labeled and hybridized onto the Rat Gene 2.1st Array strips. The reactions of hybridization, fluidics and imaging were performed on the Affymetrix Gene Atlas instrument according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA).

Blood Corticosterone Measurements at PND 62

Upon killing, trunk blood samples were collected individually in potassium EDTA tubes (1.6 mg EDTA/ml blood, Sarstedt, Germany). CORT was measured using a commercially available radioimmunoassay kit containing 125-Iodine labeled CORT (MP Biomedicals, CA). Assay sensitivity was 0.125 mg/dl. Vials were counted for 2 min in a gamma-scintillation counter (Packard Minaxi Gamma counter, Series 5000).

Statistical and Bioinformatic Analyses

Microarray data analyses: Raw microarray data (.CEL files) were imported and the expression data analysis (quality controls and statistical analyses) was performed using Partek Genomics Suite 6.6 software (Partek, St. Louis, MO, USA). Principal-component analysis (PCA) was carried out to identify possible outliers and major effects in the data. Analysis of Variance Test (ANOVA) was performed for the evaluation of differences in the gene expression levels between PNS rats and control animals at PND or in each group (PNS or control) at different PNDs. A maximum filter of $p<0.05$ and a minimum absolute fold change cut-off of 1.2 was applied to select the lists of the most significant genes.

Pathway and network analyses: The "Core Analysis" function included in IPA (Ingenuity System Inc, USA http://www.ingenuity.com) was used to understand the data in the context of biological processes, pathways and networks and upstream regulators associated with each condition of interest.

RESULTS

Biological mechanisms and pathways associated with the long-term effect of the prenatal stress (PNS) exposure.

Our first aim was to identify transcriptomic changes which occurred in adult animals (PND 62) exposed to PNS. To reach this aim we compared the transcriptomic profile in the prefrontal cortex of PNS exposed animals versus control animals. We identified a list of 389 genes whose expression profile was significantly different between PNS and control animals (226 downregulated and 163 upregulated, p-value<0.05 and Fold change±1.1, see **Supplementary Table 1**). We then performed pathway analysis to identify the biological processes involved in the long-term effects of PNS and we found that the 389 genes were enriched in 26 pathways, which mainly belong to inflammation and immune system (50%) and metabolism (34%) (**see Table 1** for the entire list of pathways). Among the most significant pathways we found the *Dendritic Cell Maturation*, *Neuroinflammation signalling pathway*, *Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) Signalling*, and *p38 MAPK signalling*. By looking at the networks involved in the modulation of the 389 genes between PNS versus CTRL, we found two significant networks which are related to the stress response and metabolism. Indeed, as we can see from the **Figure 1**, most of the genes that are connected with each other are involved in *Glucocorticoid receptor signalling*, *Leptin Signalling*, *Insulin receptor Signalling*, and *Growth Hormone Signalling*. Moreover, we also reported significantly higher corticosterone levels in PNS exposed animals as compared to control animals (control: 250±43 ng/ml, PNS: 411±62 ng/ml, n=8 animals per group).

Neurodevelopmental trajectories in control animals: transcriptomic analyses at PND 21, 40 and 62.

We were then interested in identifying a transcriptomic profile over time associated with a physiological neurodevelopment (PND 21, 40 and 62) occurring in the prefrontal cortex of these animals. To do this, we looked at changes in the transcriptomic profile in control animals at different PNDs: 21, 40 and 62. We

identified a list of 804 genes whose expression profile significantly changed between PND 40 and PND 21 (Fold change ±1.2 and p-value<0.05, 449 downregulated and 355 upregulated, see **Supplementary Table 2**) and 219 significantly modulated genes (Fold change ±1.2 and p-value<0.05, 129 downregulated and 90 upregulated, see **Supplementary Table 3**) between PND 62 and PND 40.

We then performed pathway analyses to identify the biological processes involved in the neurodevelopmental trajectories and we found that the 804 and 219 genes were enriched in 97 and 15 pathways respectively (p-value<0.05, see **Supplementary Table 4** and **Supplementary Table 5**). Among the most significant pathways modulated between PND40 and PND 21 we found the *Glycoprotein VI Platelet (GP6) Signalling Pathway*, *Actin Cytoskeleton Signalling*, *Integrin Signalling and Phosphatase* and *Tensin Homolog (PTEN) signalling*; whereas among the most significant pathways modulated between PND 62 vs 40 we found the *Growth Hormone Signalling*, *Circadian Rhythm Signalling*, *Cholesterol Biosynthesis I and Cholesterol Biosynthesis II.*

Neurodevelopmental trajectories in prenatally stressed animals: transcriptomic analyses at PND 21, 40 and 62.

We were then interested to evaluate how the physiological neurodevelopmental profile observed in the PFC of control animals could be affected by the early exposure to PNS. We thus evaluated the transcriptomic profile over time during neurodevelopment (PND 21, 40 and 62) in the PFC of PNS exposed animals. Similarly to what was done in control animals, we compared the changes in the transcriptomic profile between PND 40 versus PND 21 and those between PND 62 versus PND 40. We identified a list of 661 modulated genes whose expression profile significantly changed between PND 40 vs PND 21 (376 downregulated and 285 upregulated, see **Table 2**) in PNS animals, and 342 significantly modulated genes between PND 62 vs PND 40 (229 downregulated and 113 upregulated, **Table 3**).

We then performed pathway analyses to identify the biological processes involved in the effect of PNS in affecting the neurodevelopmental trajectories and we found that the 661 and 342 genes were enriched in 66 and 20 pathways respectively (see **Table 4** and **Table 5**). Among the most significant pathways modulated at

PND 40 versus PND 21 we found the *GP6 Signalling Pathway*, *Actin Cytoskeleton Signalling*, *Integrin Signalling and cAMP-mediated Signalling*; whereas among the most significant pathways modulated at PND 62 vs PND 40 we found *Nuclear factor erythroid 2-related factor 2 (NRF2)-mediated Oxidative Stress Response*, *Triacylglycerol Biosynthesis*, *Osteoarthritis Pathway and Liver X Receptor (LXR)/ Retinoid X Receptor (RXR) Activation.*

Transcriptomic changes specifically related to animals exposed to prenatal stress.

Our aim was then to identify transcriptomic changes during neurodevelopment (PND 21, 40 and 62) which occurred specifically in animals exposed to PNS and not in control animals. Thus, we overlapped the list of genes significantly modulated in PNS-exposed animals between PND 40 and 21 and those found modulated between PND 62 and 40, with genes significantly modulated in control animals during the same temporal window.

When considering the first temporal window (PND 40 vs 21), as we can see in the Venny Diagram (**Figure 2**) we found 444 genes modulated only in control animals (248 downregulated and 196 upregulated, see **Supplementary Table 6**), 301 genes modulated only in the PNS exposed animals (174 downregulated and 127 upregulated, see **Supplementary Table 7**) and 360 genes which resulted modulated in both groups.

We then performed pathway analyses on all the different gene lists, in order to identify the main biological processes affected by the PNS exposure. We first focused on the 444 genes modulated only in control animals and we found that they were involved in 52 biological processes (see **Table 6**) and, among the most significant pathways we found the *Glioblastoma Multiforme signalling*, *GP6 signalling Pathway*, *Actin Cytoskeleton signalling*, *PTEN signalling*.

We also looked at pathways modulated on the 301 genes which are changed only in PNS exposed animals and we found that they were enriched in 25 biological processes mainly related to inflammation (**see Table 7**). Indeed, among the most significant pathways, there are biological processes involved in inflammation and stress response (which represented the 33% of the all biological processes involved), *such as Leukocyte*

Extravasation Signalling, Integrin Linked Kinase (ILK) Signalling, NRF2-mediated Oxidative Stress Response, Dendritic Cell Maturation.

We then used a similar approach to identify changes over time, between PND 40 and 62, specifically modulated in PNS exposed animals. We integrated by Venny Diagram the genes that changed significantly between PND62 and PND40 in control animals with those that changed significantly in PNS animals. As we can see in the Venny Diagram (**Figure 3**), we found 133 genes modulated only in control animals (78 downregulated and 55 upregulated, see **Supplementary Table 8**), 256 genes modulated only in the PNS exposed animals (180 downregulated and 76 upregulated, see **Supplementary Table 9**) and 86 genes in common between the two groups.

We then performed pathway analyses, in order to identify the main biological processes affected by the PNS exposure. We found that the 133 genes which are modulated only in control animals are involved in 19 biological processes (**see Table 8**) and among the most significant pathways we found *Synaptic Long-Term Depression, Cholesterol Biosynthesis I, Cholesterol Biosynthesis II and Cholesterol Biosynthesis III*; the 256 genes which are modulated only in PNS exposed animals are mainly involved in 18 biological processes (see **Table 9**) and among the most significant pathways there are biological processes involved in metabolism, inflammation and stress response (which represented the 40% of all the involved processes), *including NRF2-mediated Oxidative Stress Response, Transforming Growth Factor Beta (TGF-β) signalling and Acute Phase Response signalling.*

Network and upstream regulators driving the effects of prenatal stress.

As we were interested in identifying biological processes and mechanisms which are altered during the neurodevelopmental window specifically in animals exposed to PNS, we also run a network analyses by focusing specifically on those genes which were modulated over time only in PNS exposed animals.

By looking at the networks mainly represented by the 301 and 256 genes respectively modulated between PND 40 and PND 21 and between PND 62 and PND 40 in PNS animals, we found networks involved in the inflammatory response and in the metabolism.

Indeed, as we can see from the network in **Figure 4a** (in PNS animals at PND 40 versus 21), most of the genes that are connected with each other are involved in *Neuroinflammation signalling pathway*, *Glucocorticoid receptor signalling* and *Acute phase response signalling*. Moreover, most of them are upregulated, suggesting that the signalling converging to inflammation and stress response is expected to be upregulated as well. Among the hub-genes/molecules within this network we can identify **NF-kB complex**, which is also a key upstream regulator of the PNS induced gene expression changes in our dataset. NF-kB complex indeed regulates several target genes, all upregulated in our database: Cluster of differentiation 74 (Cd74), Tissue inhibitor matrix metalloproteinase 1 (Timp1), B-cell lymphoma (Bcl2), Cadherin-like protein 22 (Cdh22).

In the second network represented in **Figure 4b,** most of the genes that are connected with each other are involved in *AMP-activated protein kinase (AMPK) signalling*, *Leptin signalling* and *Glucocorticoid receptor signalling*. Moreover, most of them are downregulated and related to metabolism, suggesting that the signalling converging to metabolism processes is expected to be downregulated in PNS animals. Among this network, we can identify Leptin (LEP), which is also an upstream regulator of the observed changes in gene expression in our dataset.

A similar downregulated signature could also be observed in the network in **Figure 5a** (in PNS animals at PND 62 versus 40), whereas most of the genes that are connected with each other are downregulated and involved in metabolism processes like Sirtuin Signalling Pathway, AMPK signalling and LXR/RXR Activation. Among this network, we can also identify again LEP, which is an upstream regulator of the PNS observed changes in gene expression in our dataset and a key regulator of metabolism. Interestingly, as we can see from the network in **Figure 5b** (in PNS animals at PND 62 versus 40), most of the genes that are connected with each other are involved in *Neuroinflammation signalling pathway*, *Glucocorticoid receptor signalling and Dendritic cell maturation*, suggesting that the signalling converging to inflammation and stress response is expected to be altered by PNS. Among this network, we can also identify Tumor Necrosis Factor (TNF), which is a key upstream regulator of the PNS observed changes in gene expression in our dataset. TNF as upstream regulator targets 10 genes from our database: Fos Proto-Oncogene (FOS),

Transmembrane 4 L Six Family Member 1 (TM4SF1), Nuclear Receptor Subfamily 4 Group A Member 3 (NR4A3), Cytochrome P450 Family 26 Subfamily B Member 1 (CYP26B1), Immediate Early Response 3 (IER3), Ectonucleotide Pyrophosphatase/Phosphodiesterase 3 (ENPP3), Insulin Like Growth Factor 1 (IGF1), Transgelin (TAGLN), Vimentin (VIM), Osteoglycin (OGN), most of them resulted in an activated state.

DISCUSSION

In the current study, by focusing on the effects of an early exposure to PNS in shaping the neurodevelopmental trajectories, we observe long-term alterations of pathways and networks associated with stress, the inflammatory response and metabolism, and we identify adolescence as the most critical temporal window of vulnerability for the development of stress-related psychiatric disorders. These data suggest that PNS induces alterations in several biological systems, especially in those related to stress, inflammation and metabolism, with an effect that is at its peak already during adolescence, although it is maintained until adulthood.

It is known that stressful exposures early in life, for instance during pregnancy or during childhood, shape the brain development enhancing the subsequent offspring's risk of developing mental and physical disorders later in life (Buss et al., 2012; Scheinost et al., 2017). Indeed, the developing brain (during fetal life or in childhood) is particularly plastic and thus sensitive to adverse events that may occurring during these periods (Lindsay et al., 2019). In order to study the effects of stress early in life on brain developmental trajectories, we took advantage of a well-established animal model of PNS and we analyzed the transcriptomic profile within the PFC of both control and PNS animals during neurodevelopment that is at different PNDs: 21, 40 and 62. First, we evaluated the effect of PNS in adult male animals and we found that PNS induced alterations mainly in the stress and inflammatory/immune system response, as shown by the pathway analysis, which indeed indicated *Neuroinflammation signalling pathway, NF-kB Signalling and p38 MAPK signalling* as the most significant ones. Moreover, PNS exposed rats showed higher levels of serum corticosterone, the stress hormone, as compared to their matched controls. These data are also in line with what we previously observed by measuring the transcriptomic profile in another region of the same animals,

the hippocampus. Indeed, in our previous study we identified neurodevelopment and inflammation related processes as the most significantly modulated by the PNS exposure (Cattaneo et al., 2018).

We then wanted to evaluate whether PNS exposure could affect the physiological brain trajectory during neurodevelopment; thus, we first identified the transcriptomic profiles associated with a normal brain development in control animals at different PNDs (21, 40 and 62) and then we compared these profiles with transcriptomic signatures observed in PNS exposed animals at the same PNDs. When we investigated the temporal window spanning between PND 40 to 21 and looked specifically at changes occurring in PNS exposed animals, we found alterations in the expression of 301 genes, which were mainly involved in pathways related to inflammation and metabolism, such as the *Leukocyte Extravasation Signalling*, *ILK Signalling*, *NRF2-mediated Oxidative Stress Response*, and *Dendritic Cell Maturation*.

A network analysis run on genes specifically modulated between PND 40 and PND 21 in PNS animals only, revealed two main significant networks. In the first one, most of the genes connected with each other were involved in *Neuroinflammation signalling pathway*, *Glucocorticoid receptor signalling* and *Acute phase response signalling*. Moreover, most of these genes were upregulated, suggesting an overexpression of their related processes. Interestingly, among the hub-genes/molecules within this network we identified *NF-kB complex*, which indeed regulated (up-regulation) several target genes within the network (Cd74, Timp1, Bcl2, Cdh22). In the second network, most of the genes were downregulated and were involved in *AMPK signalling*, and *Leptin signalling*. Interestingly, Leptin (downregulated), appears the hub gene of this network, meaning responsible of the modulation of most of the genes within this network.

Using a similar approach, we investigated the modulation of pathways and networks modulated specifically in PNS exposed animals during the temporal window spanning from PND 62 to PND 40. We found 256 genes specifically modulated in PNS exposed rats, most of which were again involved in biological processes related to inflammation, such as the *NRF2-mediated Oxidative Stress Response*, *TGF-β signalling* and *Acute Phase Response signalling*. The network analysis revealed, similarly to what observed within PND 40-21 two main networks. In the first one, most of the genes connected with each other were again

involved in the *Neuroinflammation signalling pathway*, *Glucocorticoid receptor signalling* and Dendritic cell maturation, and, in this network, we identified TNF, as the hub gene, which was directly involved in the modulation (activation) of several target genes in our dataset (FOS, TM4SF1, NR4A3, CYP26B1, IER3, ENPP3, IGF1, TAGLN, VIM, OGN),

In line with these data in animals, several clinical studies have demonstrated that early life stress, both prenatally and in childhood, is associated with an increased inflammatory status, in terms of high CRP levels and proinflammatory cytokines, later in life (Danese and Baldwin, 2017). This has been clearly illustrated in the prospective cohort study by Baldwin and colleagues (Baldwin et al., 2018), reporting an association between childhood victimization and elevated levels of CRP at age 18 and with the later on development of psychopathology. A growing body of literature data also suggests that immune dysregulation is present in adolescents affected by psychiatric disorders (Gabbay et al., 2009; Miklowitz et al., 2016; Mitchell and Goldstein, 2014). For example, in the study conducted by Peters and colleagues, depressed adolescents with and without a history of childhood trauma and naïve to pharmacological treatment for depression showed higher serum levels of IL-6 as compared to their matched controls (Peters et al., 2019).

Interestingly, NF-kB and TNF-alpha, which are known to play key roles in the inflammatory responses, represent the hub-genes within the two main networks identified during the temporal window between PND 40 and PND 21 and between PND 62 and PND 40 respectively. NF-kB is a transcription factor that regulates pro-inflammatory cytokines and it is implicated in a variety of cancer, autoimmune, and inflammatory disease processes. Moreover, it is activated by acute stress and it is involved in mediating cellular responses to stressful life events, which are involved in the relapse/remission course of stress-related psychiatric disorders. Indeed, an increased NF-kB activation has been suggested to lead to an activation of the inflammatory signalling, leading to an increased expression of proinflammatory cytokines and attenuated neuroendocrine responses to stress (Miklowitz et al., 2016).

Moreover, it has been showed that adolescents with a history of early life stress have increased percentages of T-cell activation markers and senescent T cells, as well as decreased percentages of natural killer (NK)

and NK T cells (Bielas et al., 2012; do Prado et al., 2017). In an interesting study, do Prado and colleagues have found that lymphocytes from adolescents with no mental disorders who were exposed to early life stress produced more pro-inflammatory cytokines, including IL-2, IL-4, IFN-γ, TNF-α and IL-17, and activated more MAPK ERK and NF-κB signalling in comparison to controls (do Prado et al., 2017). These findings suggest the presence of an enhanced immune activation and pro-inflammatory profiles in healthy adolescents exposed to early life stress, which could contribute to an increased vulnerability of stress/trauma-related psychopathology later in life (do Prado et al., 2017). Interestingly, in a recent review and meta-analysis (D'Acunto et al., 2019), higher levels of TNF-α serum have been found in depressed versus non-depressed children/adolescents.

When we looked at the pathways associated with the two different temporal windows, spanning from PND 40 to PND 21 and from PND 62 to 40 respectively, we found *NRF2-mediated Oxidative Stress Response* as a common biological process. This pathway has been largely involved in the inflammatory response, but also in the oxidative stress mechanisms (Reddy et al., 2012). Nuclear factor erythroid 2-related factor 2 (NRF2), a leucine zipper redox-sensitive transcription factor, is an important regulator of cell survivor and adaptive mechanisms. In healthy conditions, NRF2 is sequestered in the cytoplasm by a cytosolic regulatory protein, Keap1, whereas in conditions of elevated oxidative stress it translocates from cytoplasm to the nucleus and sequentially binds to a promoter sequence called the antioxidant response element (ARE), resulting in the expression of antioxidant and cytoprotective genes that have the potential to attenuate cellular damage (Reddy et al., 2012). Of note, several studies have highlighted a critical role for NRF2 in protecting the foetus during adverse *in utero* oxidative stress and damage, thereby minimizing developmental impact and long-term consequences for the offspring's health (Chapple et al., 2015). Because of its important role during early development, diminished NRF2 defences following an exposure to early life stress could predispose the offspring to oxidative damage and potentially to the onset of mental disorders in later life.

Interestingly, recently it has extensively described the presence of an increased brain oxidative damage as consequence of stress early in life (Salim, 2017). Oxidative stress, including higher production of reactive oxygen and nitrogen species (ROS and RNS), have been extensively implicated in the progression of

psychiatric disorders, due to the high vulnerability of brain to increased oxidative load (Cattane et al., 2018; Pandya et al., 2013). ROS leads to damage, either directly or indirectly, of many biological structures, including lipids, proteins and DNA, causing detrimental effects at both cellular and systemic levels (Blokhina et al., 2003). However, at moderate concentrations, the imbalance between ROS and RNS plays an important role in physiological processes, as for example in defense against infectious agents, and cellular signaling processes (Pandya et al., 2013).

In a recent study, do Prado and colleagues (do Prado et al., 2016) have hypothesized that adolescents exposed to childhood abuse and neglect may have an imbalance of ROS and antioxidant defences. They observed an important imbalance between oxidative molecules/antioxidant defences in adolescents who have undergone childhood maltreatment as compared to their matched controls. In details, they found an increased superoxide dismutase (SOD)/Glutathione Peroxidase (GPX) ratio in adolescents exposed to early life stress, suggesting that childhood maltreatment is associated with an increased oxidative stress (do Prado et al., 2016).

Besides the identification of pathways and networks associated with inflammation and oxidative stress response, we also found that most of the genes connected with each other were involved in metabolism and were downregulated. Therefore, this suggests that the signalling converging to metabolism is more likely to be downregulated. In this context, a growing body of evidence has suggested that early life stress is associated with metabolic dysfunctions later in life (Maniam et al., 2014; Yam et al., 2019). Indeed, several animal models of stress induction in pregnant females have found that their offspring are heavier and exhibit greater adiposity, impaired glycemic control, and increased food intake (Paternain et al., 2013; Paternain et al., 2012). In humans, a history of prenatal stress exposure (i.e. maternal depression, maternal bereavement, or disaster-related maternal stress during pregnancy) is associated with an increased risk of childhood obesity and type 2 diabetes, and, in young adulthood, with a higher BMI, increased percent body fat, insulin resistance, and an unfavourable lipid profile (Entringer et al., 2008). The Leptin signalling represents a clear example of a deregulation of the metabolic signalling. Indeed, among the networks, we identified Leptin as the most important downregulated hub-gene. Leptin is an adipocyte-derived hormone, which acts through receptors in the hypothalamic arcuate nucleus, resulting in reduced food intake and increased energy

expenditure (Stoving et al., 2009). However, there is also evidence that leptin regulates circuits involved in the stress response and cognition (Farr et al., 2015). Indeed, consistent with to our findings, several preclinical and clinical studies have shown that early life stress reduces leptin levels early and later in life (Danese et al., 2014; Llorente-Berzal et al., 2011; Salzmann et al., 2004; Schmidt et al., 2006). Interestingly, low leptin levels have been found to be associated with human depression and depression-like behaviours in rodents (Lu, 2007).

Another signalling that is altered already during adolescence and may represent a first signature of vulnerability for the later on development of psychiatric disorders is represented by the glucocorticoid signalling. It is well known that early in life adversities during critical periods of brain development exert a programming effect on particular neuronal networks related to the stress response and lead to enduring neuroendocrine alterations, i.e., hyper- or hypoactivation of the stress system, associated with adult hypothalamic-pituitary-adrenal axis and glucocorticoid signalling dysregulation. Neuroendocrine changes, secondary to early-life, stress likely reflect risk to develop depression in response to stress, potentially due to failure of a connected neural circuitry implicated in emotional, neuroendocrine and autonomic control to balance the responses to a challenge. Interestingly, evidences on alterations in the HPA axis with enhanced release of cortisol can be observed in adults but also in adolescents exposed to childhood trauma, suggesting that early life stress act on the HPA axis leading to a decreased responsiveness to glucocorticoids, a phenomenon known as glucocorticoid resistance, which is related in part to an impaired function of the GR (Carvalho and Pariante, 2008; Pariante, 2006; Pariante and Miller, 2001; Pariante et al., 1995).

A communication occurs between the endocrine, immune and central nervous system, where an activation of the inflammatory responses can affect neuroendocrine processes, and vice versa. Therefore, HPA axis hyperactivity and inflammation might be part of the same process: HPA axis hyperactivity is associated with glucocorticoid resistance, which implies ineffective ability of glucocorticoids to act on their targets, including the immune/inflammatory system, and as a consequence there is an enhanced activation of the immune/inflammatory response; on the other site, inflammation can stimulate HPA axis activity via both a direct action of cytokines on the brain and by inducing glucocorticoid resistance.

Interestingly, in adolescent animals exposed to PNS we observed alterations both in the glucocorticoid signalling as well as in the inflammatory processing, supporting the interplay between the two systems in

configuring a vulnerability trait already in adolescence that could predispose to the development of depression.

CONCLUSIONS

Overall our findings thus corroborate the hypothesis that adolescence represents a vulnerability temporal window in association with early life stress exposures, where changes in several biological processes are already manifested, although behavioral alterations are not manifested yet. Adolescence represents the period of life stretching between childhood and adulthood (Sawyer et al., 2018) characterized for biological and neurological changes and, for this reason, is of particular interest for the study of the neurobiological mechanisms underlying brain development. Since adolescence is marked by dramatic modifications in physical, social, cognitive and psychological behavior (Choudhury et al., 2006), it also represents one of the most vulnerable period for the development of mental disorders. In this context, our data suggest that an exposure to PNS could compromise the development of correct brain trajectories, triggering the biological basis for the future development of psychiatric disorders later in life.

These data have an important clinical implication: indeed, molecules related to the inflammatory systems, stress response and metabolism could be used as peripheral biomarkers useful in the early identification - already during adolescence - of individuals which have been exposed to adversities early in life and are more vulnerable to develop psychopathology later in life. These ‘high risk’ adolescents may benefit from preventive strategies to prevent the onset of the pathology. Moreover, novel pharmacological or non-pharmacological therapies could be used to target these biological systems in order to block or reverse the effect of stress on the individual vulnerability.

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CONFLICT OF INTEREST:

Professor Pariante has received research funding from Johnson & Johnson as part of a programme of research on depression and inflammation. In addition, Professor Pariante has received research funding from the Medical Research Council (UK) and the Wellcome Trust for research on depression and inflammation as part of two large consortia that also include Johnson & Johnson, GSK, Pfizer and Lundbeck.

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AUTHOR CONTRIBUTIONS:

NL, MM and VB contributed to the experiments in the animal model. Authors NL, MM, VZ and NC managed the literature searches and the bioinformatics and statistical analyses. NL, MM, VZ, and NC contributed to the first draft of the manuscript, MAR and CP contributed to the revision and AC revised all the versions of the manuscript and approved the final one. All the authors contributed to and have approved the final manuscript.

Table 1. List of pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 62 between control animals and those previously exposed to PNS (p-value < 0.05).

Table 2. List of 661 genes whose expression profile significantly changed at PND 40 versus PND 21 in animals previously exposed to PNS (p-value < 0.05).

Table 3. List of 342 genes whose expression profile significantly changed at PND 62 versus PND 40 in animals previously exposed to PNS (p-value < 0.05).

Table 4. List of pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 40 versus PND 21 in animals previously exposed to PNS (p-value < 0.05).

Table 5. List of pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 62 versus PND 40 in animals previously exposed to PNS (p-value < 0.05).

Table 6. List of pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 40 versus PND 21 and which occurred only in control animals (p-value < 0.05).

Table 7. List of pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 40 versus PND 21 and which occurred only in animals previously exposed to PNS (p-value < 0.05).

Table 8. pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 62 versus PND 40 and which occurred only in control animals (p-value < 0.05).

Table 9. pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 62 versus PND 40 and which occurred only in animals previously exposed to PNS (p-value < 0.05).

Figure 1. Network analyses of the genes modulated at PND 62 between control animals and those previously exposed to PNS.

Figure 2. Venny diagram to intersect the genes modulated between PND 40 and 21 in control animals and those modulated in animals previously exposed to PNS.

Figure 3. Venny diagram to intersect the genes modulated between PND 62 and 40 in control animals and those modulated in animals previously exposed to PNS

Figure 4. Network analyses of the genes modulated at PND 40 vs 21 only in animals previously exposed to PNS.

Figure 5. Network analyses of the genes modulated at PND 62 vs 40 only in animals previously exposed to PNS.

Supplementary table 1. List of 389 genes whose expression profile significantly changed at PND 62 between control animals and those previously exposed to PNS (p-value < 0.05).

Supplementary table 2. List of 804 genes whose expression profile significantly changed at PND 40 versus PND 21 in control animals (p-value < 0.05).

Supplementary table 3. List of 219 genes whose expression profile significantly changed at PND 62 versus PND 40 in control animals (p-value < 0.05).

Supplementary table 4. List of pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 40 versus PND 21 in control animals (p-value < 0.05).

Supplementary table 5. List of pathways (obtained from Ingenuity pathway analysis) differentially modulated at PND 62 versus PND 40 in control animals (p-value < 0.05).

Supplementary table 6. List of 444 genes (obtained post Venny diagram) whose expression profile significantly changed at PND 40 versus PND 21 and which occurred only in control animals (p-value < 0.05).

Supplementary table 7. List of 301 genes (obtained post Venny diagram) whose expression profile significantly changed at PND 40 versus PND 21 and which occurred only animals previously exposed to PNS (p-value < 0.05).

Supplementary table 8. List of 133 genes (obtained post Venny diagram) whose expression profile significantly changed at PND 62 versus PND 40 and which occurred only in control animals (p-value < 0.05).

Supplementary table 9. List of 256 genes (obtained post Venny diagram) whose expression profile significantly changed at PND 62 versus PND 40 and which occurred only in animals previously exposed to PNS (p-value < 0.05).

REFERENCES

Baldwin, J.R., Arseneault, L., Caspi, A., Fisher, H.L., Moffitt, T.E., Odgers, C.L., Pariante, C., Ambler, A., Dove, R., Kepa, A., Matthews, T., Menard, A., Sugden, K., Williams, B., Danese, A., 2018. Childhood victimization and inflammation in young adulthood: A genetically sensitive cohort study. Brain, behavior, and immunity 67, 211-217.

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C.M., Mondelli, V., 2016. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-alpha. Molecular psychiatry 21, 642-649.

Bengoetxea, X., Paternain, L., Martisova, E., Milagro, F.I., Martinez, J.A., Campion, J., Ramirez, M.J., 2017. Effects of perinatal diet and prenatal stress on the behavioural profile of aged male and female rats. Journal of psychopharmacology 31, 356-364.

Bielas, H., Jud, A., Lips, U., Reichenbach, J., Landolt, M.A., 2012. Increased number of activated T cells in lymphocyte subsets of maltreated children: data from a pilot study. Journal of psychosomatic research 73, 313-318.

Blokhina, O., Virolainen, E., Fagerstedt, K.V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Annals of botany 91 Spec No, 179-194.

Brunton, P.J., Russell, J.A., 2010. Prenatal social stress in the rat programmes neuroendocrine and behavioural responses to stress in the adult offspring: sex-specific effects. Journal of neuroendocrinology 22, 258-271.

Buss, C., Entringer, S., Swanson, J.M., Wadhwa, P.D., 2012. The Role of Stress in Brain Development: The Gestational Environment's Long-Term Effects on the Brain. Cerebrum : the Dana forum on brain science 2012, 4.

Carvalho, L.A., Pariante, C.M., 2008. In vitro modulation of the glucocorticoid receptor by antidepressants. Stress 11, 411-424.

Cattane, N., Richetto, J., Cattaneo, A., 2018. Prenatal exposure to environmental insults and enhanced risk of developing Schizophrenia and Autism Spectrum Disorder: focus on biological pathways and epigenetic mechanisms. Neuroscience and biobehavioral reviews.

Cattaneo, A., Begni, V., Malpighi, C., Cattane, N., Luoni, A., Pariante, C., Riva, M.A., 2019. Transcriptional Signatures of Cognitive Impairment in Rat Exposed to Prenatal Stress. Molecular neurobiology.

Cattaneo, A., Cattane, N., Malpighi, C., Czamara, D., Suarez, A., Mariani, N., Kajantie, E., Luoni, A., Eriksson, J.G., Lahti, J., Mondelli, V., Dazzan, P., Raikkonen, K., Binder, E.B., Riva, M.A., Pariante, C.M., 2018. FoxO1, A2M, and TGF-beta1: three novel genes predicting depression in gene X environment interactions are identified using cross-species and cross-tissues transcriptomic and miRNomic analyses. Molecular psychiatry 23, 2192-2208.

Cattaneo, A., Gennarelli, M., Uher, R., Breen, G., Farmer, A., Aitchison, K.J., Craig, I.W., Anacker, C., Zunsztain, P.A., McGuffin, P., Pariante, C.M., 2013. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'predictors' and longitudinal 'targets'. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 38, 377-385.

Chapple, S.J., Puszyk, W.M., Mann, G.E., 2015. Keap1-Nrf2 regulated redox signaling in utero: Priming of disease susceptibility in offspring. Free radical biology & medicine 88, 212-220.

Choudhury, S., Blakemore, S.J., Charman, T., 2006. Social cognitive development during adolescence. Social cognitive and affective neuroscience 1, 165-174.

D'Acunto, G., Nageye, F., Zhang, J., Masi, G., Cortese, S., 2019. Inflammatory Cytokines in Children and Adolescents with Depressive Disorders: A Systematic Review and Meta-Analysis. Journal of child and adolescent psychopharmacology 29, 362-369.

Danese, A., Baldwin, J.R., 2017. Hidden Wounds? Inflammatory Links Between Childhood Trauma and Psychopathology. Annual review of psychology 68, 517-544.

Danese, A., Dove, R., Belsky, D.W., Henchy, J., Williams, B., Ambler, A., Arseneault, L., 2014. Leptin deficiency in maltreated children. Translational psychiatry 4, e446.

Danese, A., Pariante, C.M., Caspi, A., Taylor, A., Poulton, R., 2007. Childhood maltreatment predicts adult inflammation in a life-course study. Proceedings of the National Academy of Sciences of the United States of America 104, 1319-1324.

De Bellis, M.D., Nooner, K.B., Scheid, J.M., Cohen, J.A., 2019. Depression in Maltreated Children and Adolescents. Child and adolescent psychiatric clinics of North America 28, 289-302.

Debost, J.P., Larsen, J.T., Munk-Olsen, T., Mortensen, P.B., Meyer, U., Petersen, L., 2017. Joint Effects of Exposure to Prenatal Infection and Peripubertal Psychological Trauma in Schizophrenia. Schizophrenia bulletin 43, 171-179.
Diz-Chaves, Y., Astiz, M., Bellini, M.J., Garcia-Segura, L.M., 2013. Prenatal stress increases the expression of proinflammatory cytokines and exacerbates the inflammatory response to LPS in the hippocampal formation of adult male mice. Brain, behavior, and immunity 28, 196-206.
Diz-Chaves, Y., Pernia, O., Carrero, P., Garcia-Segura, L.M., 2012. Prenatal stress causes alterations in the morphology of microglia and the inflammatory response of the hippocampus of adult female mice. Journal of neuroinflammation 9, 71.
do Prado, C.H., Grassi-Oliveira, R., Daruy-Filho, L., Wieck, A., Bauer, M.E., 2017. Evidence for Immune Activation and Resistance to Glucocorticoids Following Childhood Maltreatment in Adolescents Without Psychopathology. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 42, 2272-2282.
do Prado, C.H., Grassi-Oliveira, R., Wieck, A., Zaparte, A., Filho, L.D., da Silva Morrone, M., Moreira, J.C., Bauer, M.E., 2016. The impact of childhood maltreatment on redox state: Relationship with oxidative damage and antioxidant defenses in adolescents with no psychiatric disorder. Neuroscience letters 617, 173-177.
Entringer, S., Wust, S., Kumsta, R., Layes, I.M., Nelson, E.L., Hellhammer, D.H., Wadhwa, P.D., 2008. Prenatal psychosocial stress exposure is associated with insulin resistance in young adults. American journal of obstetrics and gynecology 199, 498 e491-497.
Farr, O.M., Tsoukas, M.A., Mantzoros, C.S., 2015. Leptin and the brain: influences on brain development, cognitive functioning and psychiatric disorders. Metabolism: clinical and experimental 64, 114-130.
Gabbay, V., Klein, R.G., Guttman, L.E., Babb, J.S., Alonso, C.M., Nishawala, M., Katz, Y., Gaite, M.R., Gonzalez, C.J., 2009. A preliminary study of cytokines in suicidal and nonsuicidal adolescents with major depression. Journal of child and adolescent psychopharmacology 19, 423-430.
Giovanoli, S., Engler, H., Engler, A., Richetto, J., Voget, M., Willi, R., Winter, C., Riva, M.A., Mortensen, P.B., Feldon, J., Schedlowski, M., Meyer, U., 2013. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. Science 339, 1095-1099.
Goldstein, J.M., Holsen, L., Huang, G., Hammond, B.D., James-Todd, T., Cherkerzian, S., Hale, T.M., Handa, R.J., 2016. Prenatal stress-immune programming of sex differences in comorbidity of depression and obesity/metabolic syndrome. Dialogues in clinical neuroscience 18, 425-436.
Grigoryan, G., Segal, M., 2013. Prenatal stress affects network properties of rat hippocampal neurons. Biological psychiatry 73, 1095-1102.
Grosse, L., Ambree, O., Jorgens, S., Jawahar, M.C., Singhal, G., Stacey, D., Arolt, V., Baune, B.T., 2016. Cytokine levels in major depression are related to childhood trauma but not to recent stressors. Psychoneuroendocrinology 73, 24-31.
Guan, S.Z., Ning, L., Tao, N., Lian, Y.L., Liu, J.W., Ng, T.B., 2016. Effects of maternal stress during pregnancy on learning and memory via hippocampal BDNF, Arc (Arg3.1) expression in offspring. Environmental toxicology and pharmacology 46, 158-167.
Heim, C., Mletzko, T., Purselle, D., Musselman, D.L., Nemeroff, C.B., 2008. The dexamethasone/corticotropin-releasing factor test in men with major depression: role of childhood trauma. Biological psychiatry 63, 398-405.
Holman, D.M., Ports, K.A., Buchanan, N.D., Hawkins, N.A., Merrick, M.T., Metzler, M., Trivers, K.F., 2016. The Association Between Adverse Childhood Experiences and Risk of Cancer in Adulthood: A Systematic Review of the Literature. Pediatrics 138, S81-S91.
Huffhines, L., Noser, A., Patton, S.R., 2016. The Link Between Adverse Childhood Experiences and Diabetes. Current diabetes reports 16, 54.
Jaworska-Andryszewska, P., Rybakowski, J.K., 2019. Childhood trauma in mood disorders: Neurobiological mechanisms and implications for treatment. Pharmacological reports : PR 71, 112-120.
Lahti, M., Savolainen, K., Tuovinen, S., Pesonen, A.K., Lahti, J., Heinonen, K., Hamalainen, E., Laivuori, H., Villa, P.M., Reynolds, R.M., Kajantie, E., Raikkonen, K., 2017. Maternal Depressive Symptoms During and After Pregnancy and Psychiatric Problems in Children. Journal of the American Academy of Child and Adolescent Psychiatry 56, 30-39 e37.
Laloux, C., Mairesse, J., Van Camp, G., Giovine, A., Branchi, I., Bouret, S., Morley-Fletcher, S., Bergonzelli, G., Malagodi, M., Gradini, R., Nicoletti, F., Darnaudery, M., Maccari, S., 2012. Anxiety-like

behaviour and associated neurochemical and endocrinological alterations in male pups exposed to prenatal stress. Psychoneuroendocrinology 37, 1646-1658.
Lei, M.K., Beach, S.R.H., Simons, R.L., 2018. Childhood trauma, pubertal timing, and cardiovascular risk in adulthood. Health psychology : official journal of the Division of Health Psychology, American Psychological Association 37, 613-617.
Lindsay, K.L., Buss, C., Wadhwa, P.D., Entringer, S., 2019. The Interplay Between Nutrition and Stress in Pregnancy: Implications for Fetal Programming of Brain Development. Biological psychiatry 85, 135-149.
Llorente-Berzal, A., Fuentes, S., Gagliano, H., Lopez-Gallardo, M., Armario, A., Viveros, M.P., Nadal, R., 2011. Sex-dependent effects of maternal deprivation and adolescent cannabinoid treatment on adult rat behaviour. Addiction biology 16, 624-637.
Lu, S., Gao, W., Huang, M., Li, L., Xu, Y., 2016. In search of the HPA axis activity in unipolar depression patients with childhood trauma: Combined cortisol awakening response and dexamethasone suppression test. Journal of psychiatric research 78, 24-30.
Lu, X.Y., 2007. The leptin hypothesis of depression: a potential link between mood disorders and obesity? Current opinion in pharmacology 7, 648-652.
Luoni, A., Berry, A., Raggi, C., Bellisario, V., Cirulli, F., Riva, M.A., 2016. Sex-Specific Effects of Prenatal Stress on Bdnf Expression in Response to an Acute Challenge in Rats: a Role for Gadd45beta. Molecular neurobiology 53, 7037-7047.
Luoni, A., Richetto, J., Longo, L., Riva, M.A., 2017. Chronic lurasidone treatment normalizes GABAergic marker alterations in the dorsal hippocampus of mice exposed to prenatal immune activation. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 27, 170-179.
Mandelli, L., Petrelli, C., Serretti, A., 2015. The role of specific early trauma in adult depression: A meta-analysis of published literature. Childhood trauma and adult depression. European psychiatry : the journal of the Association of European Psychiatrists 30, 665-680.
Maniam, J., Antoniadis, C., Morris, M.J., 2014. Early-Life Stress, HPA Axis Adaptation, and Mechanisms Contributing to Later Health Outcomes. Frontiers in endocrinology 5, 73.
Mayo, D., Corey, S., Kelly, L.H., Yohannes, S., Youngquist, A.L., Stuart, B.K., Niendam, T.A., Loewy, R.L., 2017. The Role of Trauma and Stressful Life Events among Individuals at Clinical High Risk for Psychosis: A Review. Frontiers in psychiatry 8, 55.
Miklowitz, D.J., Portnoff, L.C., Armstrong, C.C., Keenan-Miller, D., Breen, E.C., Muscatell, K.A., Eisenberger, N.I., Irwin, M.R., 2016. Inflammatory cytokines and nuclear factor-kappa B activation in adolescents with bipolar and major depressive disorders. Psychiatry research 241, 315-322.
Mitchell, R.H., Goldstein, B.I., 2014. Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. Journal of the American Academy of Child and Adolescent Psychiatry 53, 274-296.
Muller, N., Krause, D., Barth, R., Myint, A.M., Weidinger, E., Stettinger, W., Zill, P., Drexhage, H., Schwarz, M.J., 2019. Childhood Adversity and Current Stress are related to Pro- and Anti-inflammatory Cytokines in Major Depression. Journal of affective disorders 253, 270-276.
Osborne, S., Biaggi, A., Chua, T.E., Du Preez, A., Hazelgrove, K., Nikkheslat, N., Previti, G., Zunszain, P.A., Conroy, S., Pariante, C.M., 2018. Antenatal depression programs cortisol stress reactivity in offspring through increased maternal inflammation and cortisol in pregnancy: The Psychiatry Research and Motherhood - Depression (PRAM-D) Study. Psychoneuroendocrinology 98, 211-221.
Palmisano, G.L., Innamorati, M., Vanderlinden, J., 2016. Life adverse experiences in relation with obesity and binge eating disorder: A systematic review. Journal of behavioral addictions 5, 11-31.
Pandya, C.D., Howell, K.R., Pillai, A., 2013. Antioxidants as potential therapeutics for neuropsychiatric disorders. Progress in neuro-psychopharmacology & biological psychiatry 46, 214-223.
Pariante, C.M., 2006. The glucocorticoid receptor: part of the solution or part of the problem? Journal of psychopharmacology 20, 79-84.
Pariante, C.M., Miller, A.H., 2001. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biological psychiatry 49, 391-404.
Pariante, C.M., Nemeroff, C.B., Miller, A.H., 1995. Glucocorticoid receptors in depression. Israel journal of medical sciences 31, 705-712.
Paternain, L., de la Garza, A.L., Batlle, M.A., Milagro, F.I., Martinez, J.A., Campion, J., 2013. Prenatal stress increases the obesogenic effects of a high-fat-sucrose diet in adult rats in a sex-specific manner. Stress 16, 220-232.

Paternain, L., Martisova, E., Milagro, F.I., Ramirez, M.J., Martinez, J.A., Campion, J., 2012. Postnatal maternal separation modifies the response to an obesogenic diet in adulthood in rats. Disease models & mechanisms 5, 691-697.
Paul, E., Eckenrode, J., 2015. Childhood psychological maltreatment subtypes and adolescent depressive symptoms. Child abuse & neglect 47, 38-47.
Peters, A.T., Ren, X., Bessette, K.L., Goldstein, B.I., West, A.E., Langenecker, S.A., Pandey, G.N., 2019. Interplay between pro-inflammatory cytokines, childhood trauma, and executive function in depressed adolescents. Journal of psychiatric research 114, 1-10.
Pfeffer, C.R., Altemus, M., Heo, M., Jiang, H., 2007. Salivary cortisol and psychopathology in children bereaved by the september 11, 2001 terror attacks. Biological psychiatry 61, 957-965.
Plant, D.T., Pawlby, S., Sharp, D., Zunszain, P.A., Pariante, C.M., 2016. Prenatal maternal depression is associated with offspring inflammation at 25 years: a prospective longitudinal cohort study. Translational psychiatry 6, e936.
Reddy, P.V., Agudelo, M., Atluri, V.S., Nair, M.P., 2012. Inhibition of nuclear factor erythroid 2-related factor 2 exacerbates HIV-1 gp120-induced oxidative and inflammatory response: role in HIV associated neurocognitive disorder. Neurochemical research 37, 1697-1706.
Reynolds, R.M., Labad, J., Buss, C., Ghaemmaghami, P., Raikkonen, K., 2013. Transmitting biological effects of stress in utero: implications for mother and offspring. Psychoneuroendocrinology 38, 1843-1849.
Salim, S., 2017. Oxidative Stress and the Central Nervous System. The Journal of pharmacology and experimental therapeutics 360, 201-205.
Salzmann, C., Otis, M., Long, H., Roberge, C., Gallo-Payet, N., Walker, C.D., 2004. Inhibition of steroidogenic response to adrenocorticotropin by leptin: implications for the adrenal response to maternal separation in neonatal rats. Endocrinology 145, 1810-1822.
Sawyer, K.M., Zunszain, P.A., Dazzan, P., Pariante, C.M., 2018. Intergenerational transmission of depression: clinical observations and molecular mechanisms. Molecular psychiatry.
Scheinost, D., Sinha, R., Cross, S.N., Kwon, S.H., Sze, G., Constable, R.T., Ment, L.R., 2017. Does prenatal stress alter the developing connectome? Pediatric research 81, 214-226.
Schmidt, M.V., Levine, S., Alam, S., Harbich, D., Sterlemann, V., Ganea, K., de Kloet, E.R., Holsboer, F., Muller, M.B., 2006. Metabolic signals modulate hypothalamic-pituitary-adrenal axis activation during maternal separation of the neonatal mouse. Journal of neuroendocrinology 18, 865-874.
Simons, S.S.H., Zijlmans, M.A.C., Cillessen, A.H.N., de Weerth, C., 2019. Maternal prenatal and early postnatal distress and child stress responses at age 6. Stress, 1-10.
Stoving, R.K., Andries, A., Brixen, K., Flyvbjerg, A., Horder, K., Frystyk, J., 2009. Leptin, ghrelin, and endocannabinoids: potential therapeutic targets in anorexia nervosa. Journal of psychiatric research 43, 671-679.
Szuran, T.F., Pliska, V., Pokorny, J., Welzl, H., 2000. Prenatal stress in rats: effects on plasma corticosterone, hippocampal glucocorticoid receptors, and maze performance. Physiology & behavior 71, 353-362.
Toffol, E., Rantalainen, V., Lahti-Pulkkinen, M., Girchenko, P., Lahti, J., Tuovinen, S., Lipsanen, J., Villa, P.M., Laivuori, H., Hamalainen, E., Kajantie, E., Pesonen, A.K., Raikkonen, K., 2019. Infant regulatory behavior problems during first month of life and neurobehavioral outcomes in early childhood. European child & adolescent psychiatry 28, 847-859.
Tursich, M., Neufeld, R.W., Frewen, P.A., Harricharan, S., Kibler, J.L., Rhind, S.G., Lanius, R.A., 2014. Association of trauma exposure with proinflammatory activity: a transdiagnostic meta-analysis. Translational psychiatry 4, e413.
Van den Bergh, B.R.H., van den Heuvel, M.I., Lahti, M., Braeken, M., de Rooij, S.R., Entringer, S., Hoyer, D., Roseboom, T., Raikkonen, K., King, S., Schwab, M., 2017. Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. Neuroscience and biobehavioral reviews.
Yam, K.Y., Schipper, L., Reemst, K., Ruigrok, S.R., Abbink, M.R., Hoeijmakers, L., Naninck, E.F.G., Zarekiani, P., Oosting, A., Van der Beek, E.M., Lucassen, P.J., Korosi, A., 2019. Increasing availability of omega-3 fatty acid in the early-life diet prevents the early-life stress-induced cognitive impairments without affecting metabolic alterations. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 33, 5729-5740.
Zeugmann, S., Buehrsch, N., Bajbouj, M., Heuser, I., Anghelescu, I., Quante, A., 2013. Childhood maltreatment and adult proinflammatory status in patients with major depression. Psychiatria Danubina 25, 227-235.