Molecular Neurobiology (2019) 56:6251–6260
https://doi.org/10.1007/s12035-019-1523-4

Transcriptional Signatures of Cognitive Impairment in Rat Exposed to Prenatal Stress

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Received: 9 November 2018 / Accepted: 1 February 2019 / Published online: 11 February 2019

Abstract

Exposure to adverse events during gestation has detrimental effects on the maturation of specific brain networks, triggering changes in the expression of several stress-related mechanisms that may lead to long-lasting functional consequences, including cognitive deterioration. On these bases, the aim of the present study was to investigate the effects of early-life stress exposure on cognition and to explore potential molecular mechanisms contributing to the long-term functional impairment. We found that exposure to prenatal stress, a well-established animal model of early-life adversity, produces a significant disruption in the novel object recognition test both in male and female adult rats, although such impairment was more pronounced in females. Furthermore, the cognitive dysfunction observed during the behavioral test appears to be sustained by a disrupted activation of key networks of genes that may be required for proper cognitive performance. In particular, within the dorsal hippocampus, a brain region critical for cognition, the glucocorticoid, the inflammatory, and the protein kinase A signaling pathways are regulated by the novel object recognition test in an opposite manner in animals previously exposed to prenatal stress, when compared with control animals. These data further support the evidence that early-life stress exposure prompts cognitive impairment and suggest that this is the consequence of inability to activate the proper transcriptional machinery required for the cognitive performance.

Keywords Prenatal stress · Cognitive impairment · Dorsal hippocampus · Transcriptional signature

Introduction

Exposure to stress represents a well-established risk factor for the development of psychiatric disorders. In particular, stress occurring early in life appears to have more severe and protracted consequences when compared with similar events occurring during adulthood, possibly because exposure to stress during this time may alter brain maturation, leading to long-term functional changes in different brain structures [1–3]. In line with this possibility, exposure to stress during gestation can produce an array of molecular and functional changes that are associated with an enhanced risk of developing behavioral and emotional problems [4–6]. The investigation of the long-term consequences of early-life stress (ELS) on neurodevelopmental trajectories is therefore highly relevant to identify mechanisms and pathways associated with brain dysfunction in mental disorders [7, 8].

Animal models are a key tool to investigate the effects of ELS, because the timing and intensity of stress exposure can be precisely controlled [9, 10]. Prenatal stress (PNS) in rodents is a well-characterized paradigm of ELS, which relies on the exposure of pregnant dams to stress during the last week of gestation [11–13]. PNS exposure produces a host of behavioral and functional alterations that resemble some of the biological and clinical features observed in depressed patients, including a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [14], the emergence of anxiety- and depressive-like phenotypes, and deficits in neuronal plasticity [5]. For example, we have demonstrated that the expression of brain-derived neurotrophic factor (BDNF), a neurotrophin that plays a key role in neuronal maturation as well as in neuronal adaptation to environmental conditions

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12035-019-1523-4) contains supplementary material, which is available to authorized users.

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[15, 16], is significantly reduced in the prefrontal cortex (PFC) of adult rats that are exposed to PNS in utero [12]. Moreover, PNS-exposed rats show an impaired ability to respond to challenging conditions during adulthood, with associated epigenetic mechanisms [13].

One important transdiagnostic problem in mental illness is the presence of cognitive impairments, which has a strong impact on patient function and that represents a major target for drug intervention [17]. Considering, as mentioned above, that exposure to ELS represents a risk factor for the development of mood disorders later in life, it may be hypothesized that early exposure to such adverse conditions may lead to persistent cognitive dysfunctions along with other behavioral deficits. In line with this possibility, it has been shown that exposure to ELS, both prenatally as well as during the early postnatal phase, produces long-lasting alterations in cognitive function [18–23], an effect that is mimicked by glucocorticoid exposure during pregnancy [19] and that may be restored by exposure to an enriched environment after weaning [23].

In the present study, we investigate the cognitive outcome of PNS, including the potential differences between male and female animals. Furthermore, we employed a genome-wide strategy to investigate the molecular signatures associated with the cognitive performance following PNS, in order to identify systems and pathways that may be differentially regulated in response to a cognitive task (novel object recognition) in animals exposed and not exposed to PNS.

Materials and Methods

Animals and Experimental Paradigms

Pregnant rats were randomly assigned to control (CTRL) or PNS conditions. The stress paradigm was carried out as previously described [12]. Briefly, PNS consisted of restraining pregnant dams in a transparent Plexiglas cylinder under bright light for 45 min three times daily during the last week of gestation until delivery. PNS sessions were separated by 2–3-h intervals and conducted at varying periods of the day in order to reduce habituation. CTRL rats were left undisturbed.

On postnatal day (PND) 1, pups from CTRL and PNS dams were weighed, and litters were culled to five males and five females. Weaning occurred on PND21, and same sex rats were housed in groups of three per cage and left undisturbed until adulthood.

In details, at PND73 (males) and at PND80 (females), half of the rats pertaining to both CTRL and PNS groups was tested in the novel object recognition (NOR) test (CTRL/NOR ($n = 14$) and PNS/NOR ($n = 15$) groups), whereas the other half was left undisturbed in their home cages until sacrifice. Female rats were killed 30 min after the end of the behavioral test, and the brain region of interest (the dorsal hippocampus) was immediately dissected, frozen on dry ice, and kept at − 80 °C for the further molecular analyses.

All animal experiments were conducted according to the authorization from the Health Ministry n. 295/2012-A (20/12/2012), in full accordance with the Italian legislation on animal experimentation (Decreto Legislativo 116/92) and adherent to EU recommendation (EEC Council Directive 86/609), in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

All efforts were made to minimize animal suffering and reduce the total number of animals used, while maintaining statistically valid group numbers.

No pre-established inclusion/exclusion criteria were used for the subsequent molecular analyses. All samples were processed and analyzed by investigators blind to the PNS or behavioral conditions.

Object Recognition Test Procedure

The animals were tested in a non-transparent open field (43 × 43 × 32 cm) made of Plexiglas, placed in a quiet room dimly illuminated.

On the day of testing, animals were habituated in the room for a 30-min period, before the experimental procedure began. As previously reported for Sprague-Dawley rats [24–26], the experiment comprised two sessions of 300 s each. During the first session (familiarization phase), two identical objects were presented to the animal. During an inter-trial interval of 3 min, which the rats spent in their home cages, one of the two familiar objects was replaced by a novel, previously unseen object (with distinctive different shape, color, and texture). Rats were returned to the open field and presented to the familiar and novel objects during the second session of 300 s (test phase). For both sessions, object exploration time (i.e., sitting in close proximity to the objects, sniffing, or touching them) was manually measured by a trained observer (blind to the experimental conditions), and a discrimination index was calculated for each animal and expressed as follows:

$$[(\text{time novel object} - \text{time familiar object}) / (\text{time novel object} + \text{time familiar object})] \times 100$$

After the end of the test phase, rats were returned to their home cages and sacrificed by decapitation 30 min later. The testing cage was wiped clean with 0.1% acetic acid and dried after each trial.

Total RNA Preparation

Total RNA was isolated from the rat dorsal hippocampus using PureZol RNA isolation reagent (Bio-Rad Laboratories, Italy) according the manufacturer's instructions and processed for microarray gene expression analyses. An aliquot of each sample was treated with DNase to avoid DNA contamination and quantified by spectrophotometric analysis.

Microarray Gene Expression Analyses

Gene expression microarray assays were performed as reported in our previous works [27]. The analyses were performed using Rat Gene 2.1ST Array Strips on GeneAtlas TM platform (Affymetrix, Santa Clara, CA, USA), following the WT Expression Kit protocol as described in the "Affymetrix GeneChip Expression Analysis Technical Manual."

Briefly, starting from 250 ng of total RNA, cDNA was synthetized with the GeneAtlas WT Expression Kit (Affymetrix, Santa Clara, CA, USA). The concentration and quality of cRNA and cDNA were determined by measuring its absorbance at 260 nm using a NanoDrop spectrophotometer. After fragmentation and labeling procedures, 5.5 μg of cDNA were hybridized using a Rat Gene 2.1 ST Array Strip. The hybridization, the fluidics, and the imaging were performed on the Affymetrix Gene Atlas instrument according to the manufacturer's protocol. All the samples were randomized, processed, and analyzed by experimenters' blind to the paradigm group.

Statistical and Bioinformatic Analyses

Changes produced by PNS exposure in the NOR test were analyzed with Student's *t* test. For microarray data analyses, Affymetrix .CEL files were imported into Partek Genomics Suite version 6.6 (Partek, St. Louis, MO, USA) for data visualization, quality control, and statistical testing. All samples passed the criteria for hybridization controls, labeling controls, and 3′/5′ metrics. Background correction was conducted using Robust Multi-strip Average (RMA) to remove noise from autofluorescence. After background correction, normalization was conducted using quantile normalization [28] to normalize the distribution of probe intensities among different microarray chips. Subsequently, a summarization step was conducted using a linear median polish algorithm to integrate probe intensities in order to compute the expression levels for each gene transcript. After the pre-processing of .CEL files for quality control, we aimed to investigate the effect of the PNS and NOR test exposure and their combination. Thus, we first included in the two-way ANOVA the two main independent variables (PNS and NOR), allowing us to assess their impact in the whole sample. Subsequently, we applied three contrasts (PNS/SHAM vs CTRL/SHAM; CTRL/NOR vs CTRL/SHAM; PNS/NOR vs PNS/SHAM) in order to get the transcriptomic profiles in each specific condition of interest. In these comparisons, a filter of a p value of < 0.05 and of fold change (FC) cutoff of 1.2 was applied to get lists of significant genes. Genes that passed these criteria were used to run further analyses. In particular, Ingenuity Pathway Analyses (IPA) software was used to identify a significant regulation of molecular signaling pathways and networks in each condition. In this case, we kept a significance threshold of a log value equal to 1.3 ($p < 0.05$).

Results

Working Memory Deficits in Prenatally Stressed Rats

To determine whether PNS can impair cognitive function, the NOR test was performed in both male and female adult rats, who had been exposed to PNS, compared with control animals.

PNS exposure led to a disruption in the NOR, as measured as discrimination index. Rats of both genders, regardless of PNS exposure, showed no preference for either of the two identical objects presented during the familiarization phase, since they spent the same amount of time exploring the two familiar objects (data not shown). As expected, during the test phase, control animals effectively remembered the familiar object, as they spent significantly more time exploring the novel object compared with the familiar one (males: $t(6) = -4.935$, $p < 0.01$; females: $t(18) = -4.214$, $p < 0.001$, Fig. 1a). However, animals exposed to PNS showed a significant impairment in the ability to differentiate the two objects, as shown by the significant reduction of the discrimination index; this impairment was more severe in females than males (males: $t(8) = 3.049$, $p < 0.05$; females: $t(17) = 3.954$, $p < 0.001$, Fig. 1b).

Comparative Transcriptomic Profile After NOR Test in Control or PNS-Exposed Female Rats

Next, we wanted to investigate the biological mechanisms that might contribute to the cognitive deficit of PNS rats in the NOR test. In order to identify gene expression changes and pathway modulation in association with the cognitive impairment, we focused on female rats as they had shown the largest impairment following PNS exposure. The analysis was performed in the dorsal hippocampus, a region critical for cognitive function [29].

In order to achieve this aim, we performed transcriptomic analyses in four groups of animals: (i) control animals not exposed to the NOR test (CTRL_SHAM), (ii) control animals exposed to the NOR test (CTRL_NOR), (iii) animals exposed to PNS but not to the NOR test (PNS_SHAM), and (iv) animals exposed to PNS and to the NOR test (PNS_NOR). Our analyses allowed the identification of (i) gene expression changes associated with PNS exposure only, (ii) gene expression changes associated with the NOR test in control animals,

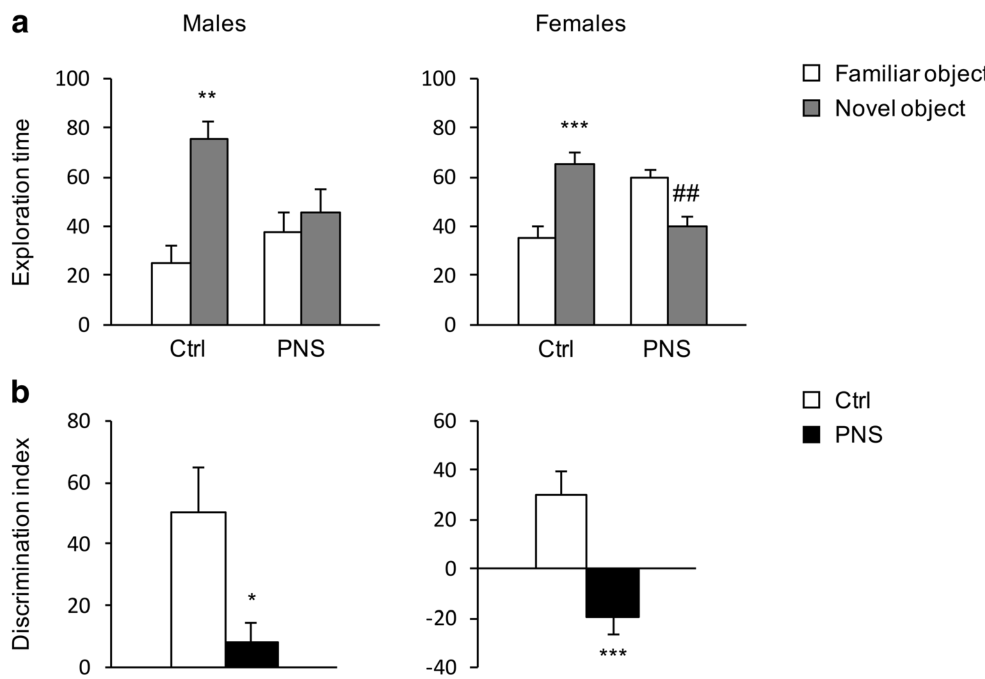

Fig. 1 Effect of prenatal stress (PNS) on cognitive performances as measured with the novel object recognition test in male and female rats at adulthood (PND73 and PND80, respectively). **a** Percentage of exploration time of the familiar vs the novel object during the test phase. Each value represents the mean ± SEM of at least 4 animals per group. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs CTRL/familiar object; $^{\#\#}p < 0.01$ vs PNS/familiar object (Student's *t* test). **b** Discrimination index calculated as the difference between time spent exploring novel and familiar objects during the test phase. Each value represents the mean ± SEM of at least 4 animals per group. $^{*}p < 0.05$ and $^{***}p < 0.001$ vs CTRL animals (Student's *t* test)

and (iii) gene expression changes associated with the NOR test in PNS animals.

By using a value of 1.2 as FC cutoff and $p < 0.05$ for significance, we found that PNS per se altered the expression of 63 genes, 32 up-regulated and 31 down-regulated in the absence of NOR (see Online Resource 1, Supplementary Table 1), which are involved in the modulation of 16 significant pathways (p value < 0.05), including Th1 and Th2 activation pathway, T helper cell differentiation, IL-7 signaling pathway, mineralocorticoid biosynthesis, and glucocorticoid biosynthesis as the top significant pathways (the list of all the significant pathways is reported in Online Resource 1, Supplementary Table 2). More importantly, we found that exposure to the NOR test produced different gene expression profiles in control animals compared with those exposed to PNS (Fig. 2). In particular, in the dorsal hippocampus, we found that exposure to the NOR test produced significant changes in 513 genes (308 up-regulated and 205 down-regulated) in CTRL rats and in 170 genes (135 up-regulated and 35 down-regulated) in rats exposed to PNS (see Online Resource 1, Supplementary Tables 3 and 4 for list of genes modulated by NOR in CTRL animals and by NOR in PNS animals).

By using a Venn diagram, we were able to identify genes that were modulated by the NOR test specifically in CTRL animals or in PNS-exposed animals and to identify also the genes shared between the two groups. As we can see from Fig. 3, the pattern of changes was highly different between CTRL and PNS rats, since 451 genes were specifically modulated by NOR test selectively in CTRL animals (88% of all regulated genes in this experimental group), 108 specifically in PNS rats (64% of all regulated genes in this experimental group), whereas only 62 genes were regulated in both groups.

We first focused the attention on the 451 genes specifically modulated in CTRL animals exposed to the NOR test that were not regulated by NOR in PNS-exposed rats in order to identify the pathways or networks whose modulation could contribute to the cognitive performance. Hence, we ran a pathway analysis by using IPA software, and we found the significant modulation of 38 biological signaling pathways (see Online Resource 1, Supplementary Table 5 for the entire list), including the EIF2 signaling, p38 MAPK signaling, T helper cell differentiation, HMGB1 signaling, neuroinflammation signaling, MIF-mediated glucocorticoid regulation, and other pathways that are associated with neuroplasticity, such as Notch signaling, PKA signaling, NGF signaling, and PI3K/AKT signaling.

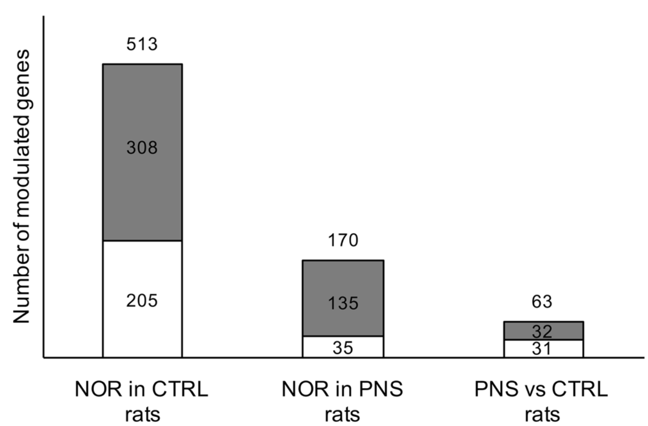

Fig. 2 Gene expression changes (expressed in term of number of modulated genes) as consequence of NOR test in CTRL animals in PNS-exposed animals and effect of PNS only. Gray indicates the up-regulated genes; white indicates the down-regulated genes

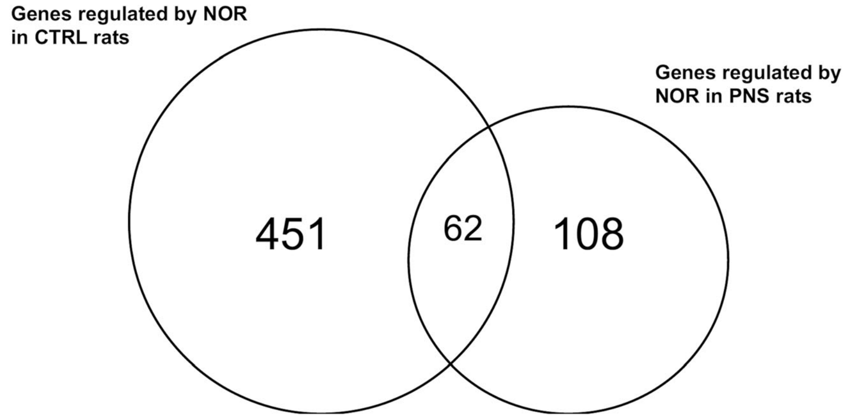

Fig. 3 Venn diagram to intersect the genes modulated by NOR in CTRL animals and by NOR in PNS-exposed animals. 451 = genes modulated by NOR specifically in CTRL animals; 108 = number of genes modulated by NOR specifically in PNS-exposed animals; 62 = genes in common as modulated by NOR both in CTRL and in PNS-exposed animals

We next looked at gene networks to better investigate the modulation of these genes and to establish the connections within specific pathways. The results of this analysis are reported in Fig. 4, where the top four networks are shown. Interestingly, as we can see from *network 1*, most of the genes that are connected with each other are involved in EIF2 signaling. Moreover, most of them are down-regulated, suggesting that the signaling converging to E2F signaling is expected to be down-regulated as well. Among this network, we can also identify MYCN, which is also the main upstream regulator of the observed changes in gene expression in our dataset. MYCN is a transcription factor, which regulates

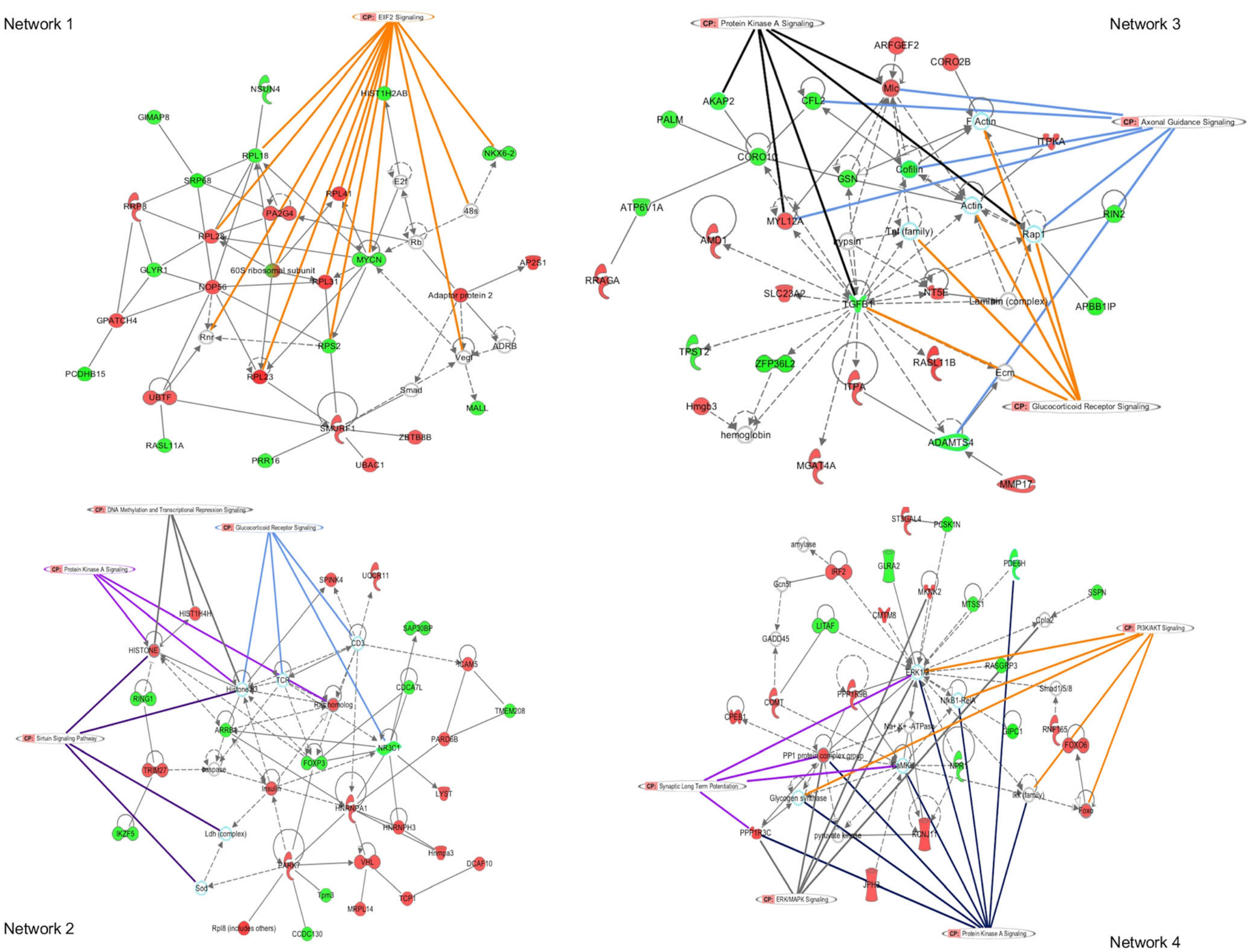

Fig. 4 Representation of the most significant networks and of the related signaling involved in the effect of NOR in CTRL animals. Green indicates genes, which are down-regulated, and red indicates genes that are up-regulated. Networks have been identified by using IPA software

several target genes (ITGB1, ABCC1, CAV1, TP53, HMGA1, MXI1, focal adhesion kinase, ITGA3, ITGA2, HDAC2, CCND1, NME1, RPL10, TIMP2, MDM2, SP1, E2F3, SOX2). MYCN is down-regulated in animals exposed to NOR test, and its down-regulation may lead also to the down-regulation of all these MYCN-regulated genes and their related signaling.

In *network 2*, we found genes involved in glucocorticoid signaling, sirtuin signaling, protein kinase A (PKA) signaling, DNA methylation, and transcriptional repression signaling. The glucocorticoid receptor (GR) is down-regulated, suggesting a down-regulation of its signaling; HISTONE and HIST1H4H are up-regulated, suggesting an up-regulation of the DNA methylation and transcriptional repression pathways, whereas HISTONE and Ras are up-regulated, suggesting an up-regulation of the PKA signaling.

Networks 3 and *4* reported genes that are primary involved in neuroplasticity, such as not only PKA signaling as in network 2 but also axonal guidance, synaptic long-term potentiation (LTP), ERK/MAPK signaling, and PI3K/AKT signaling. Most of these genes are up-regulated (Mlc, MYL12A, FoxO, FoxO6, PP1 protein complex, PPP1R3C), suggesting a potential activation of neuroplasticity-related mechanisms in association with the "positive" performance during the NOR test.

We then focused on the genes that were modulated by NOR only in animals previously exposed to PNS ($n = 108$ genes), and we found significant changes in 29 pathways ($p < 0.05$, see Online Resource 1, Supplementary Table 6), primarily involved in inflammation, GR signaling, and neuroplasticity, which are similar to the pathways modulated by the NOR test in CTRL animals. However, we found an opposite modulation of these signaling pathways, as confirmed also in the network analyses. Indeed, we found that the interactions between the 108 genes are represented by two main networks (represented in Fig. 5). Genes in *network 1* are primarily involved in stress response and in neuroplasticity, with an activation of the glucocorticoid signaling, as suggested by an up-regulation of FKBP-5, SMARCD2, and TGF-β and an inactivation of PKA signaling, cAMP signaling, and synaptic LTP, as suggested by a down-regulation of ADCY and CAMK4.

Network 2 is primarily involved in inflammation, as the genes are related to the signaling of IL-6, HMGB1, Th1, Th2, and GR. Interestingly, all these pathways were up-regulated following NOR exposure, and the genes belonging to these processes, such as pro-inflammatory cytokines Sox11 and NFIL3, were also induced by NOR (shown in red in the picture).

Finally, we focused the attention on the 62 genes modulated by NOR both in CTRL animals and PNS-exposed animals. Among these 62 genes, 56 genes were modulated in the same direction, whereas only 6 genes (NPAS4, Ppif, Cotl, Ift20, Stk16, and Naa20) were regulated in opposite directions (see Online Resource 1, Supplementary Table 7 for details of FC). We then ran a network analysis on the 56 common genes modulated in the same direction in CTRL and PNS rats. Theoretically, considering the different performances in the NOR test in the two groups of rats, these genes should not participate to the behavioral outcome. Interestingly, most of these genes were up-regulated and appeared to be related to stress response as well as to neuronal activation, which may represent a consequence of the environmental exposure to the test arena. Indeed, we found the involvement of three main networks (Fig. 6), with an activation of several pathways, including the glucocorticoid signaling, NfKb signaling, inflammation, PKA signaling, and ERK/MAPK signaling. In

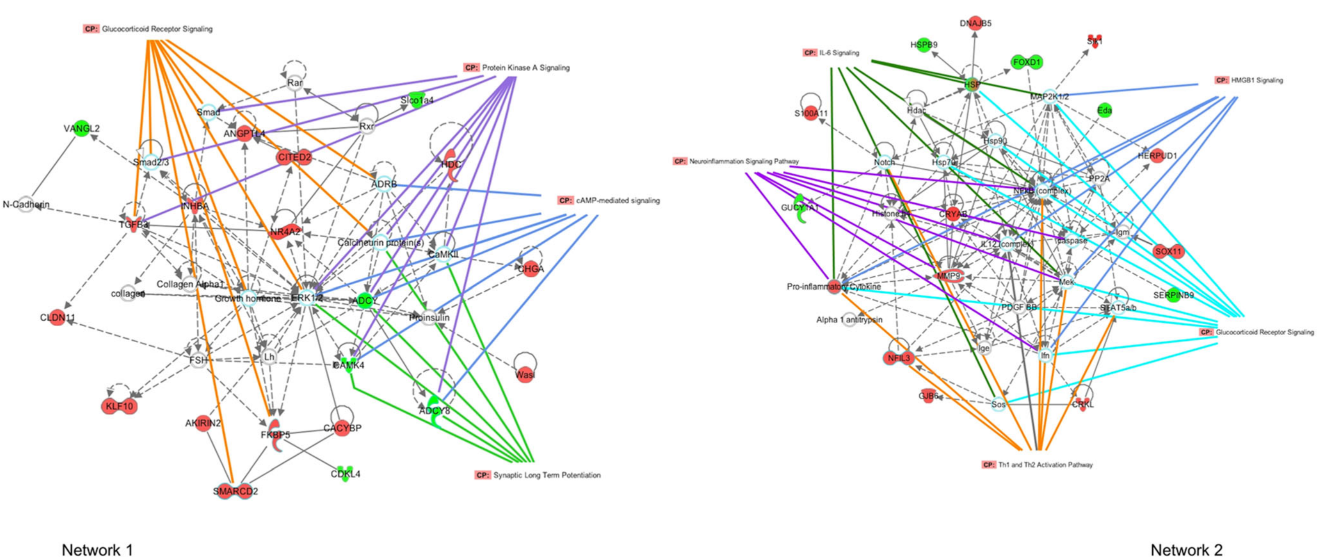

Fig. 5 Representation of the most significant networks and of the related signaling involved in the effect of NOR in PNS-exposed animals. Green indicates genes, which are down-regulated, and red indicates genes that are up-regulated. Networks have been identified by using IPA software

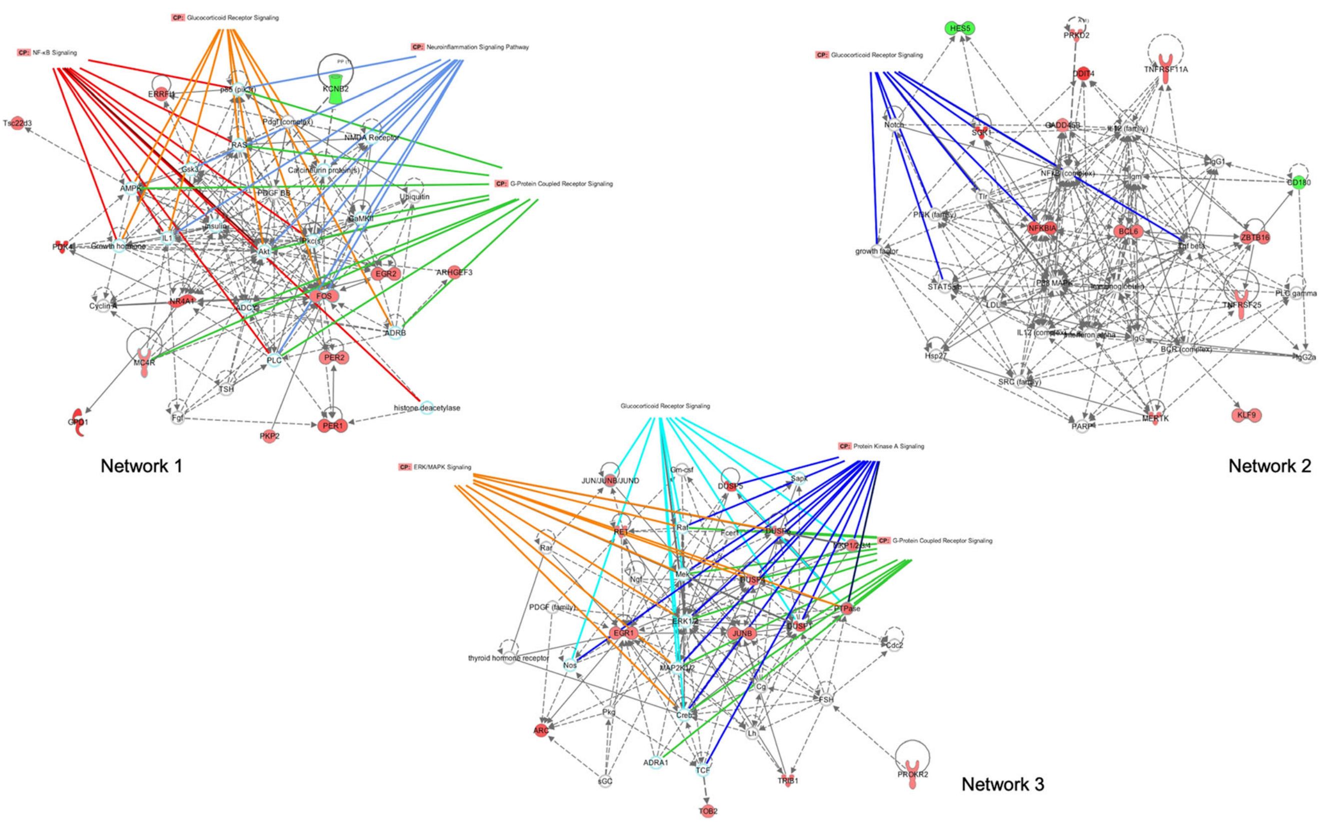

Fig. 6 Representation of the most significant networks and of the related signaling involved in the effect of NOR both in CTRL as well as in PNS-exposed animals. Green indicates genes, which are down-regulated, and red indicates genes that are up-regulated. Networks have been identified by using IPA software

the latter groups, there are Arc and Zif-268 (Egr-1) that represent prototypical inducible early genes and are considered markers of neuronal activation.

Discussion

Compelling evidence supports the idea that exposure to stress early in life (ELS) represents a major element of vulnerability for the development of psychiatric disorders later in life [30–32]. In line with clinical studies, animals exposed to stressful experience during gestation as well as in the early phase of their postnatal life show a number of behavioral changes that recapitulate specific psychiatric domains, including anhedonia, anxiety, and cognitive impairment [18–23, 33, 34]. Studies have tried to identify the molecular underpinnings of such long-term dysfunction, using targeted as well as genome-wide approaches [35].

The results of our study provide further support to the notion that exposure to ELS leads to cognitive deficit, an effect that may be associated with a significant impairment of hippocampal synaptic plasticity [23]. It is known that PNS exposure produces long-lasting consequences in the expression and function of different biological systems relevant to cognition and brain plasticity. We have previously demonstrated that exposure to PNS produces a significant down-regulation in the expression of neurotrophic factors, including the neurotrophin BDNF and fibroblast growth factor 2 (FGF-2) [12, 13, 36]. Furthermore, a similar manipulation regulates the expression of key glutamatergic players, including the *N*-methyl-D-aspartate (NMDA) receptor subunits, NR2a, NR2b, and the scaffolding postsynaptic density protein 95 (PSD-95), in adolescent male offspring, an effect that is associated with reduced cognitive flexibility in the Morris water maze test [18].

We suggest that the functional alterations originating from stress exposure in utero may represent the consequence of an inability to activate the proper transcriptional machinery required for the performance during the cognitive test. Our work suggests that exposure to stress during pregnancy represents a priming event that leads to persistent alterations of inter-related pathways, eventually interfering with the correct and physiological response during a cognitive task. The cognitive impairment—as detected in the NOR test—was found in adult male as well as in female rats born from mothers that were exposed to PNS during the last week of gestation, suggesting that such alteration represents a common long-lasting trait for both genders. These findings are in line with previous reports that have consistently shown that exposure to adverse experiences during gestation (or early postnatal life) leads to long-lasting cognitive dysfunction [18, 20, 21, 23].

We next used a transcriptomic approach to identify genes and pathways that may play a relevant role in the behavioral deficits emerging as a consequence of PNS exposure. The analysis, in the dorsal hippocampus of female rats, shows a significantly higher number of genes modulated by NOR in control animals than in PNS rats, suggesting that the behavioral deficits observed as a consequence of the PNS experience may be due to the inability to activate key networks of genes that may contribute to the correct cognitive performance. However, the difference between CTRL and PNS rats is not only quantitative but also qualitative. Indeed, one striking evidence that emerges from the comparison between CTRL and PNS rats exposed to the NOR test is represented by two key pathways that appear to be regulated in an opposite manner: the glucocorticoid/inflammatory signaling and the PKA signaling. It has been previously demonstrated that PNS may exacerbate the effects of an acute stress on hippocampal LTP, supporting the idea that the gestational manipulation may lead to a disruption in mechanisms that are activated during the behavioral performance [23]. In particular, PNS-exposed animals, which were then exposed to NOR test, show enhanced response to the glucocorticoid/inflammatory signaling. The dysregulation of the HPA axis as a consequence of exposure to ELS represents a well-established trait in rodent models as well as in humans, as indicated by an elevation of circulating corticosterone levels, under resting conditions or following an acute stress [37]. As an example, we and others have previously shown that PNS rats display increased peripheral blood concentrations of corticosterone [14, 38]. Furthermore, offspring exposed to noise stress during gestation had more elevated levels of corticosterone than control rats, and these HPA axis changes correlated with the changes in cognitive abilities and synaptic activity [21].

However, much less is known about changes in mechanisms that are downstream from GR activation, a step that coordinates the response of other intracellular pathways activated by stress [39]. For example, we have previously shown that the up-regulation of the neurotrophin BDNF, following an acute swim stress, is impaired in rats that are exposed to stress during gestation. Such alteration is gender-specific, indicating that PNS-exposed female rats are unable to activate mechanisms that may be relevant for "coping" under challenging conditions [13]. The present results suggest that GR-related networks are down-regulated by the NOR test in CTRL animals, whereas glucocorticoid signaling is activated by NOR in PNS rats. PKA signaling regulation in response to NOR is also different in CTRL and PNS rats, with an effective activation found in control animals and an inactivation of such pathway in PNS animals. It is known that several intracellular systems associated with the activation of PKA, including the cyclic AMP responsive element binding protein (CREB) and the mitogen-activated protein kinase (MAPK), play an important role in learning and memory [40–42], which suggests that the inability to activate such pathways may contribute to the cognitive impairment observed in PNS rats. This observation is in agreement with an impaired expression of extracellular signal-regulated kinase (ERK)-cyclic AMP responsive element binding protein (CREB) signaling following exposure to PNS [43–45].

Lastly, while a limited number of genes shares a similar modulation between CTRL and PNS rats exposed to NOR, most of them appears to be associated with stress response as well as neuronal activation and not directly relevant to the cognitive task. In line with this possibility, we have previously demonstrated that an up-regulation of the activity-regulated cytoskeleton-associated gene (*Arc*) was observed following NOR exposure independently from the behavioral performance [46]. This suggests that the behavioral test may lead to a similar recruitment of specific brain structures, such as the dorsal hippocampus, although the response to such activation may diverge in terms of regulation of the transcriptional machinery and intracellular signaling mechanisms.

One limitation of our study is that we cannot exclude that the long-term consequences of PNS on cognition may be due to alterations in maternal care of dams exposed to stress during gestation. Indeed, it has been demonstrated that exposure to PNS leads to reduced maternal care [47], which may have persistent effects not only on HPA axis function but also on hippocampal dependent learning and memory [22].

In summary, our data demonstrate that exposure to PNS produces long-lasting cognitive impairment, which is associated with an inability to activate the proper transcriptional machinery in relevant brain structures during a cognitive task, such as the dorsal hippocampus. Future studies should investigate whether, in order to normalize defective behavior and cognitive function following ELS, pharmacological and non-pharmacological intervention may correct the transcriptional imbalance affecting key mechanisms, such as inflammation and PKA signaling.

Acknowledgements We thank Francesca Calabrese for her contribution in the initial part of the work.

Funding Information This work has been supported by the Italian Ministry of Instruction, University and Research (PRIN grant number 2015SKN9YT), Fondazione CARIPLO (grant number 2012-0503), and European Union/NEURON-ERANET to M.A.R. Moreover, A.C. received support from Ricerca Corrente (Ministry of Health) and from a NEURON-ERANET grant.

Compliance with Ethical Standards

All animal experiments were conducted according to the authorization from the Health Ministry n. 295/2012-A (20/12/2012), in full accordance with the Italian legislation on animal experimentation (Decreto Legislativo 116/92) and adherent to EU recommendation (EEC Council Directive 86/609), in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

All efforts were made to minimize animal suffering and reduce the total number of animals used, while maintaining statistically valid group numbers.

Conflict of Interest The author M.A.R. has received compensation as speaker/consultant from Lundbeck, Otzuka, Dainippon Sumitomo Pharma, and Sunovion, and he has received research grants from Lundbeck, Dainippon Sumitomo Pharma, and Sunovion. All the other authors declare that they have no financial interest or potential conflicts of interest.

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