OXFORD

International Journal of Neuropsychopharmacology (2018) 21(9): 883–893

doi:10.1093/ijnp/pyy046
Advance Access Publication: May 18, 2018
Regular Research Article

Regular Research Article

Chronic Stress Exposure Reduces Parvalbumin Expression in the Rat Hippocampus through an Imbalance of Redox Mechanisms: Restorative Effect of the Antipsychotic Lurasidone

Andrea C. Rossetti, Maria Serena Paladini, Martina Colombo, Piotr Gruca, Magdalena Lason-Tyburkiewicz, Katarzyna Tota-Glowczyk, Mariusz Papp, Marco A. Riva, Raffaella Molteni

Department of Medical Biotechnology and Translational Medicine (Dr Rossetti, Ms Paladini, and Dr Molteni), and Department of Pharmacological and Biomolecular Sciences (Ms Colombo and Dr Riva), University of Milan, Milan, Italy; Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland (Mr Gruca, Ms Lason-Tyburkiewicz, Ms Tota-Glowczyk, and Dr Papp).

Correspondence: Marco A. Riva, PhD, Department of Pharmacological and Biomolecular Sciences, University of Milan, Via Balzaretti, 9 - 20133 Milan, Italy (m.riva@unimi.it).

Abstract

Background: Psychiatric disorders are associated with altered function of inhibitory neurotransmission within the limbic system, which may be due to the vulnerability of selective neuronal subtypes to challenging environmental conditions, such as stress. In this context, parvalbumin-positive GABAergic interneurons, which are critically involved in processing complex cognitive tasks, are particularly vulnerable to stress exposure, an effect that may be the consequence of dysregulated redox mechanisms.
Methods: Adult Male Wistar rats were subjected to the chronic mild stress procedure for 7 weeks. After 2 weeks, both control and stress groups were further divided into matched subgroups to receive chronic administration of vehicle or lurasidone (3 mg/kg/d) for the subsequent 5 weeks. Using real-time RT-PCR and western blot, we investigated the expression of GABAergic interneuron markers and the levels of key mediators of the oxidative balance in the dorsal and ventral hippocampus.
Results: Chronic mild stress induced a specific decrease of parvalbumin expression in the dorsal hippocampus, an effect normalized by lurasidone treatment. Interestingly, the regulation of parvalbumin levels was correlated to the modulation of the antioxidant master regulator NRF2 and its chaperon protein KEAP1, which were also modulated by pharmacological intervention.
Conclusions: Our findings suggest that the susceptibility of parvalbumin neurons to stress may represent a key mechanism contributing to functional and structural impairments in specific brain regions relevant for psychiatric disorders. Moreover, we provide new insights on the mechanism of action of lurasidone, demonstrating that its chronic treatment normalizes chronic mild stress-induced parvalbumin alterations, possibly by potentiating antioxidant mechanisms, which may ameliorate specific functions that are deteriorated in psychiatric patients.

Keywords: stress, hippocampus, parvalbumin, lurasidone, NRF2

Received: February 21, 2018; **Revised:** May 2, 2018; **Accepted:** May 15, 2018

Significance Statement

Our study provides evidence for an association between the stress-induced decrease of parvalbumin, a marker of GABAergic interneurons, and the alterations of key players of the oxidative balance. Of particular interest is the observation that chronic treatment with the antipsychotic drug lurasidone is able to normalize the decrease of parvalbumin in parallel with potential antioxidative effects on the NRF2-KEAP1 system as well as on the enzyme NOX2. Our data suggest a close relationship between the alterations of selected GABAergic interneurons and the redox balance that may represent an important mechanism through which lurasidone may ameliorate brain function in stress-related pathologic conditions.

Introduction

Psychiatric diseases, such as major depression and schizophrenia, are highly disabling disorders characterized by complex etiological mechanisms that lead to functional abnormalities of different neurotransmitters, including monoamines, GABA and glutamate, as well as a dysregulation of inflammation, neuroplasticity, and hormonal signaling (Kupfer et al., 2012; Calabrese et al., 2016a; Owen et al., 2016; Begni et al., 2017). The multifaceted behavioral symptomatology of these disorders involves the perturbation of emotional and cognitive domains of the individual and, among the others, cognitive symptoms have a dramatic impact on the everyday life of the patients (Millan et al., 2012). In this context, at the cortical and hippocampal levels, the GABAergic inhibitory tone finely regulates the firing of principal glutamatergic neurons. In more detail, GABAergic interneurons synchronize the firing of principal cells controlling the plasticity of excitatory synaptic inputs through dendritic inhibition, while they inhibit the output with perisomatic inhibition (Freund, 2003). Among the diverse subtypes of GABAergic interneurons populating the hippocampal formation, parvalbumin (PVB), somatostatin (SST), calbindin (CALB), and neuropeptide-Y (NPY) positive cells represent the most sensitive to stress exposure (Filipovic et al. 2013; Czeh et al. 2015). Specifically, the highly energized, fast-spiking, PVB positive (PVB+) interneurons play a pivotal role in the processing of complex information. Their contribution in cognitive decline may be fundamental, especially when dysregulation of the energy demand and/or of the oxidative balance may impair their functions (Kann, 2016). This may occur following exposure to stress, which represents a major environmental condition for mental disorders (Pittenger and Duman, 2008; Cattaneo and Riva, 2016). Indeed, PVB+ neurons can be part of a critical loop since, while stress may lead to an impairment of this neuronal population (Zaletel et al., 2016), the suppressed function of PVB+ neurons may reduce resilience (Perova et al., 2015).

In the present work we used the chronic mild stress (CMS) model (Willner, 2017) to investigate the detrimental effects of stress on PVB+ cells in rat hippocampus and the potential contribution of a dysregulation in redox mechanisms, which have also been associated with the pathophysiology of several psychiatric disorders (Moniczewski et al., 2015; Smaga et al., 2015; Steullet et al., 2017). We have previously shown that CMS is able to induce depressive-like behaviors such as anhedonia (Rossetti et al., 2016) as well as cognitive impairment (Calabrese et al., 2017), which are associated with alterations in key molecular players for psychiatric disorders (Luoni et al., 2014; Calabrese et al., 2016b; Molteni et al., 2016). We also investigated the effect of a chronic treatment with lurasidone in counteracting the CMS-induced alterations in rat hippocampus. Lurasidone is a multi-receptor antipsychotic drug (Tarazi and Riva, 2013) with demonstrated clinical efficacy for cognitive deficits in schizophrenia (Harvey et al., 2013) and in bipolar disorder (Yatham et al., 2017) and depressive symptoms in schizophrenia (Nasrallah et al., 2015) and in bipolar depression (Loebel et al., 2014). We previously demonstrated that chronic lurasidone is able to normalize the stress-induced depressive-like behaviors as well as the neuroplastic and inflammatory alterations observed in stressed rats (Luoni et al., 2014; Rossetti et al., 2016).

Methods

Animals

Adult male Wistar rats (Charles River) were brought into the laboratory 1 month before the start of the experiment. Except as described below, the animals were singly housed with food and water freely available and were maintained on a 12-h- light/-dark cycle in a constant temperature (22±2°C) and humidity (50±5%) conditions. All procedures used in this study conform to the rules and principles of the 2010/63/EU Directive and were approved by the Local Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland. All efforts were made to minimize animal suffering and to reduce the number of animals used (n=10 each experimental group).

CMS Procedure and Pharmacological Treatment

After a period of adaptation to laboratory and housing conditions, the animals (220±7 g) were subjected to 7 weeks of CMS in parallel with a 5-week-long treatment with lurasidone.

The stress regimen consisted of 2 periods of food or water deprivation, 2 periods of 45° cage tilt, 2 periods of intermittent illumination (lights on and off every 2 h), 2 periods of soiled cage (250 mL water in sawdust bedding), one period of paired housing, 2 periods of low intensity stroboscopic illumination (150 flashes/min), and 3 periods of no stress. All stressors were 10 to 14 hours of duration and were applied individually and continuously, day and night. Control animals were housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for 14 hours preceding each sucrose test, but otherwise food and water were freely available in the home cage.

Animals were subjected to the stress procedure for 7 weeks. Following the first 2 weeks of stress, both control and stress groups were further divided into matched subgroups, and for the subsequent 5 weeks they received oral administration (by gavage) of vehicle (hydroxy-ethyl-cellulose 1%) or lurasidone (3 mg/kg daily). Our experimental design included 4 groups of animals: unstressed rats that received the vehicle, used as reference control group (“No Stress/Vehicle”, n=10); unstressed rats that received the drug (No Stress/Lurasidone, n=10); stressed rats that received the vehicle (Stress/Vehicle, n=10); and stressed rats that received the drug (Stress/Lurasidone, n=10). After 5 weeks, the treatments were terminated, and all control and stressed animals were killed by decapitation

24 hours after the last drug administration. The brains were removed and dissected for prefrontal cortex, dorsal, and ventral hippocampus as fresh tissues. All samples were then rapidly frozen in dry ice/isopentane and stored at –80°C for further molecular analyses.

RNA Preparation and Quantitative Real-Time PCR Analyses

Total RNA was isolated by single step guanidinium isothiocyanate/phenol extraction using PureZol RNA isolation reagent (Bio-Rad Laboratories S.r.l.) according to the manufacturer's instructions and quantified by spectrophotometric analysis. The samples were processed for PCR as previously described (Rossetti et al., 2016) to measure the mRNA expression of *Pvb*, *Sst*, *Calb*, *Npy*, NADPH oxygenase 2 (*Nox2*), nuclear factor (erythroid-derived 2)-like 2 (*Nrf2*), sulfiredoxin (*Srxn*), hemeoxigenase-1 (*Ho-1*), NAD(P)H dehydrogenase [quinone]1 (*Nqo1*), and catalase (*Cat*). Primer and probe sequences are listed in Table 1.

Specifically, RNA aliquots of each sample were treated with DNase to avoid DNA contamination and then analyzed by TaqMan qRT-PCR instrument (CFX384 real-time system, Bio-Rad Laboratories) using the iScript one-step RT-PCR kit for probes (Bio-Rad Laboratories). The samples were run in 384-well formats in triplicate as multiplexed reactions with a normalizing internal control (β-Actin).

Thermal cycling was initiated with an incubation at 50°C for 10 minutes (RNA retro-transcription) and then at 95°C for 5 minutes (TaqMan polymerase activation). After this initial step, 39 cycles of PCR were performed. Each PCR cycle consisted of heating the samples at 95°C for 10 seconds to enable the melting process and then for 30 seconds at 60°C for the annealing and extension reaction. A comparative cycle threshold (Ct) method was used to calculate the relative target gene expression vs the control group. Specifically, fold change for each target gene relative to β-actin was determined by the 2-Δ(ΔCT) method, where ΔCT = CT(target)-CT(β-actin); Δ(ΔCT) = ΔCT(exp. group)-ΔCT(control group); CT is the threshold cycle. For graphical clarity, the obtained data were then expressed as percentage vs control group, which has been set at 100%.

Protein Extraction and Western-Blot Analyses

Brain samples were manually homogenized using a glass-glass potter in a pH 7.4 cold buffer (containing 0.32 M sucrose, 0.1 mM EGTA, 1 mM HEPES solution, and 0.1 mM phenylmethylsulfonyl fluoride in presence of a complete set of proteases [Roche] and phosphatase [Sigma-Aldrich] inhibitors) and then sonicated for 10 seconds at a maximum power of 10% to 15% (Bandelin Sonoplus). The homogenate was clarified (1000 g; 10 minutes), obtaining a pellet (P1) enriched in nuclear components, which was resuspended in a buffer (1 mM HEPES, 0.1mM dithiothreitol, 0.1 mM EGTA) supplemented with protease and phosphatase inhibitors. The supernatant (S1) was then centrifuged (13 000g; 15 minutes) to obtain a clarified fraction of cytosolic proteins (S2). The pellet (P2), corresponding to the crude membrane fraction, was resuspended in the same buffer used for the nuclear fraction. Total protein content was measured according to the Bradford Protein Assay procedure (Bio-Rad Laboratories), using bovine serum albumin as calibration standard.

Protein analyses were performed in the whole homogenate (for PVB), in the cytosolic fraction (for NRF2 and KEAP1), and in the crude membrane fraction (for NOX2). Equal amounts of protein (10 μg for the homogenate, 30 μg for the S2, and 15 μg for the P2) were run under reducing conditions on polyacrylamide gels and then electrophoretically transferred onto polyvinylidene fluoride or nitrocellulose membranes. Unspecific binding sites were blocked with 10% nonfat dry milk; then the membranes were incubated overnight with the primary antibodies and for 1 hour at room temperature with a peroxidase-conjugated anti-rabbit or anti-mouse IgG (Table 2). Immunocomplexes were visualized by chemiluminescence using the ECL Star (Euroclone), ECL Plus (Euroclone), or ECL Clarity (Bio-Rad Laboratories). Results were standardized using β-actin as the internal control, which was detected by evaluating the band density at 43 kDa. Protein levels were calculated by measuring the optical density of the immunocomplexes using chemiluminescence (Chemidoc MP Imaging System, Bio-Rad Laboratories). To ensure that autoradiographic bands would be in the linear range of intensity, different exposure times were used.

Statistical Analyses

The effects of drug treatment (lurasidone) and chronic stress exposure on the mRNA or protein levels of our molecular targets were analyzed by 2-way ANOVA followed, when appropriate, by Fisher's Least Significant Difference (LSD) posthoc comparisons. In addition, to evaluate the association between the modulation of the NRF2-KEAP1 system and the protein levels of PVB, Pearson product-moment correlation coefficients (r) were calculated between NRF2 or KEAP1 protein and PVB levels. Significance for all tests was assumed for $P < .05$. Data are

Table 1. Sequences of Forward and Reverse Primers and Probes used in qRT-PCR Analysis

Gene	Forward Primer	Reverse Primer	Probe
*Pvalb**	CTGGACAAAGACAAAAGTGGC	GACAAGTCTCTGGCATCTGAG	CCTTCAGAATGGACCCCAGCTCA
*Sst**	ACTCCGTCAGTTTCTGCAG	CAGGGCATCGTTCTCTGTC	AGTTCCTGTTTCCCGGTGGCA
*Calb**	AGAACTTGATCCAGGAGCTTC	CTTCGGTGGGTAAGACATGG	TGGGCAGAGAGATGATGGGAAAATAGGA
*Npy**	GACAGAGATATGGCAAGAGATCC	CTAGGAAAAGTCAGGAGAGCAAG	CCCCAGAACAAGGCTTGAAGACCC
*Nrf2***	Rn00582415_m1		
*Keap1***	Rn01448220_m1		
*Nox2***	Rn00675098_m1		
*Srnx***	Rn04337926_g1		
*Ho-1***	Rn00561387_m1		
*Nqo1***	Rn00566528_m1		
*Cat***	Rn00560930_m1		
*ß Actin**	CACTTTCTACAATGAGCTGCG	CTGGATGGCTACGTACATGG	TCTGGGTCATCTTTTCACGGTTGGC

Purchased from Eurofins MWG Operon (Germany)* and Applied Biosystem (Italy)**.

Table 2. Primary and Secondary Antibodies Used in Western-Blot Analyses

Primary Antibody	Primary Antibody Condition	Secondary Antibody Condition
PVB, 10 kDa (Abcam)	1:2500 in 5% nonfat dry milk 4°C o/n	1:2500, anti-rabbit (Cell Signaling) 5% nonfat dry milk, 1h, RT
NRF2, 110 kDa (R&D System)	1:500 in 5% BSA 4°C o/2n	1:500, anti-mouse (Sigma-Aldrich) 5% BSA, 1h, RT
KEAP1, 66 kDa (R&D System)	1:250 3% nonfat dry milk 4°C o/n	1:500, anti-mouse (Sigma-Aldrich) 5% nonfat dry milk, 1h, RT
NOX2, 58 kDa (BD)	1:500 3% nonfat dry milk 4°C o/n	1:500, anti-mouse (Sigma-Aldrich) 3% nonfat dry milk, 1h, RT
β-ACTIN 43 kDa (Sigma-Aldrich)	1:2000 in 3% nonfat dry milk 1h, RT	1:20000, anti-mouse (Sigma-Aldrich) 3% nonfat dry milk, 1h, RT

Abbreviations: BSA, bovine serum albumin; o/n, overnight; o/2n, over 2 nights; RT, room temperature.

presented as means ± SEM. SPSS (Release 24.0.0.0) was used to perform the statistical analyses.

RESULTS

Analysis of the mRNA Levels for Different Subtypes of GABAergic Interneurons in Rats Exposed to CMS and Treated with Lurasidone

We first investigated the mRNA levels of the GABAergic markers *Pvb*, *Sst*, *Npy*, and *Calb* in the dorsal (D-HIP) and ventral (V-HIP) hippocampus of animals exposed to CMS and treated, or not, with the antipsychotic drug lurasidone.

The analysis of *Pvb* gene expression in the D-HIP showed a significant interaction between CMS and lurasidone treatment ($F_{3,32}$=11.755, P<.01). Indeed, stress exposure led to a significant decrease of *Pvb* mRNA levels (-18% vs No Stress/Vehicle, P<.05; Figure 1A), which was normalized by pharmacological intervention (+19% vs Stress/Vehicle, P<.05; Figure 1A). Of note, lurasidone administration per se produced a significant decrease of *Pvb* compared with control rats (-16% vs No Stress/Vehicle, P<.05; Figure 1A). These changes appeared to be specific for the dorsal part of the hippocampus, since no significant changes were found in the ventral counterpart (Figure 1E).

When investigating *Sst* expression in the D-HIP, we found a significant effect of CMS exposure ($F_{3,35}$=10.023, P<.01) as well as of pharmacological treatment ($F_{3,35}$=33.850, P<.001). As shown in Figure 1B, *Sst* levels were increased in rats subjected to CMS (+58% vs No Stress/Vehicle, P<.01; Figure 1B), whereas chronic lurasidone treatment upregulated *Sst* expression in nonstressed rats (+98% vs No Stress/Vehicle, P<.001; Figure 1B) as well as in stressed animals (+75% vs Stress/Vehicle, P<.001; Figure 1B). In the V-HIP, we found a main effect of CMS on *Sst* mRNA levels ($F_{3,36}$=6.687, P<.05) that led to a significant decrease of this marker in stressed rats compared with control animals (-21% vs No Stress/Vehicle, P<.05; Figure 1F), an effect that was not modulated by the pharmacological treatment.

Conversely, the expression of the other GABAergic markers, namely *Npy* (Figure 1C, G) and *Calb* (Figure 1D, H), was not significantly modulated in the dorsal or ventral portion of the hippocampus following CMS exposure or lurasidone treatment, providing further support to the selectivity exerted by CMS exposure on specific subpopulations of GABAergic neurons.

No significant changes were observed in the prefrontal cortex of stressed rats treated or not with chronic pharmacological treatment (Table 3).

Analysis of PVB Protein Levels in the Hippocampus of Animals Exposed to CMS and Treated with Lurasidone

Based on the gene expression analyses of different interneuron markers, we decided to focus on PVB and analyzed its protein levels in D-HIP and V-HIP of rats exposed to CMS, with or without lurasidone treatment. Within D-HIP, as depicted in Figure 2A, we found a main effect of CMS exposure ($F_{3,35}$=12.164, P<.001) and lurasidone treatment ($F_{3,35}$=19.860, P<.001) as well as a significant CMS x treatment interaction ($F_{3,35}$=7.710, P<.01). Indeed, the levels of PVB were markedly reduced in rats exposed to CMS and treated with vehicle (-58% vs No Stress/Vehicle, P<.001; Figure 2A), whereas chronic lurasidone treatment was able to normalize the CMS-induced changes of PVB levels (+67% vs Stress/Vehicle, P<.001; Figure 2A).

In line with the gene expression data, these alterations show anatomical selectivity. Indeed, within the V-HIP (Figure 2B), despite a main effect of CMS exposure ($F_{3,35}$=9.486, P<.01), we only found a trend toward a decrease of PVB levels in stressed animals (-16% vs No Stress/Vehicle P=.054), which was not influenced by the pharmacological treatment.

Analysis of NADPH Oxidase-2 Gene and Protein Expression in the Dorsal Hippocampus of Animals Exposed to CMS and Treated with Lurasidone

PVB neurons show a sustained firing activity that requires a high demand of energy, which may expose them to an increased susceptibility toward the detrimental effects of oxidative stress. On these bases, we investigated if the effects of CMS exposure in D-HIP could be associated with alterations of molecules involved in the complex machinery regulating the oxidative balance in the brain. We analyzed the gene expression of *Nox2*, an enzyme responsible for the production of reactive oxygen species by activated macrophages, including microglia. The analysis of *Nox2* mRNA levels in D-HIP revealed a significant interaction between stress and lurasidone administration ($F_{37,3}$=4.521, P<.05). Indeed, the direct comparisons between groups showed that *Nox2* gene expression increased in rats exposed to CMS (+33% vs No Stress/Vehicle, P<.05; Figure 3A), an effect that was not present in CMS rats chronically treated with lurasidone. We then investigated the protein levels of $gp91^{PHOX}$, the main membrane-bound subunit of the enzyme. As depicted in Figure 3B, stress exposure had a main effect on NOX2 protein levels ($F_{33,3}$=7,121 P<.05), since CMS rats showed an increase of NOX2 compared with control animals (+98% vs No Stress/Vehicle, P<.01; Figure 3B), an effect that was attenuated by chronic lurasidone administration.

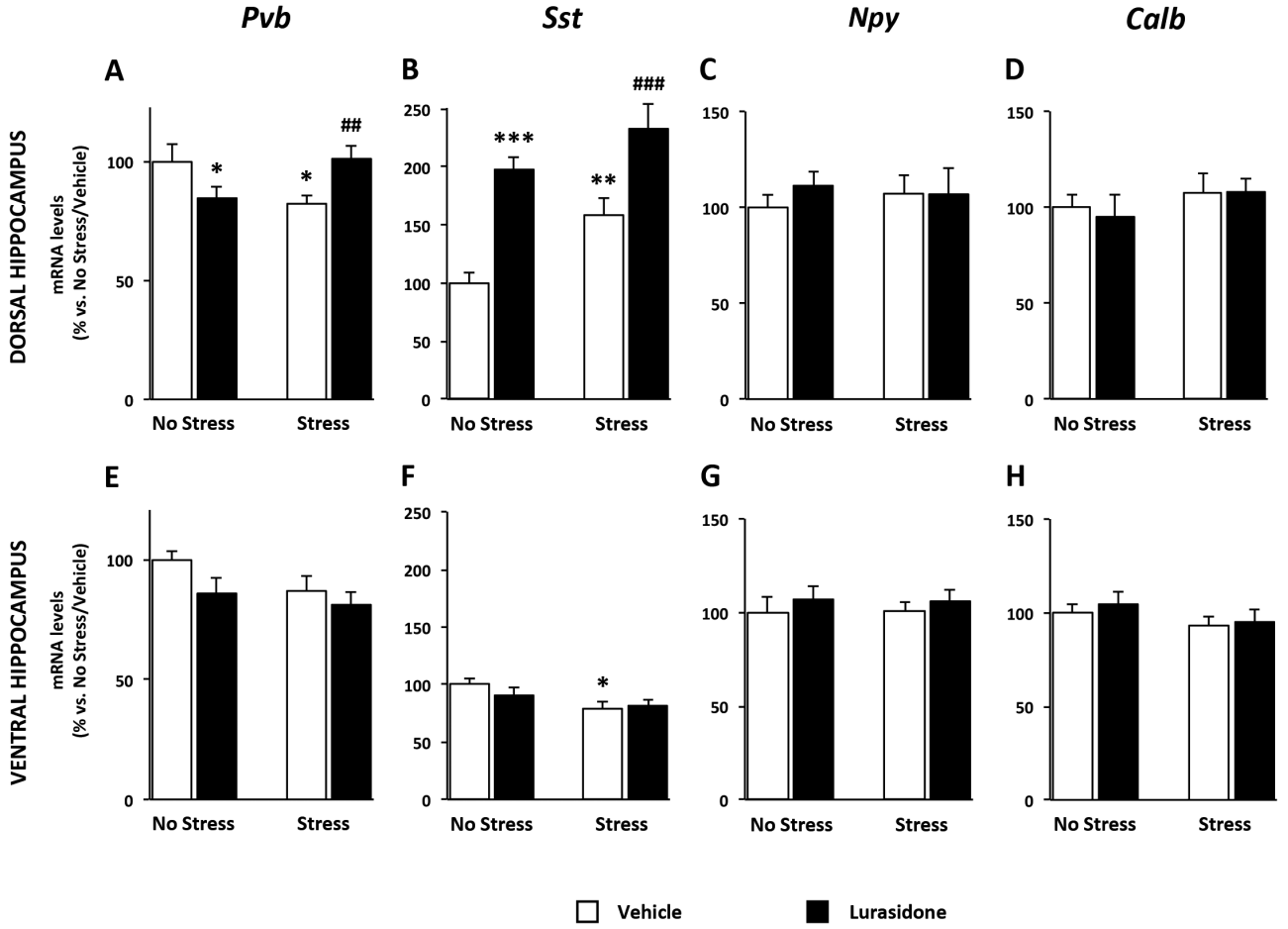

Figure 1. Gene expression analysis of interneuron markers parvalbumin (*Pvb*), somatostatin (*Sst*), neuropeptide y (*Npy*), and calbindin (*Calb*) in the dorsal and ventral hippocampus of rats exposed to chronic mild stress (CMS): modulation by lurasidone treatment. The mRNA levels of *Pvb*, *Sst*, *Npy*, and *Calb* were measured in the dorsal (A–D) and ventral (E–H) hippocampus of rats exposed to CMS in combination with chronic treatment with vehicle or lurasidone. The data, expressed as a percentage of unstressed rats treated with vehicle (No Stress/Vehicle set at 100%), are the mean +/- SEM of at least 7 independent determinations. *$P<.05$, **$P<.01$, ***$P<.001$ vs No Stress/Vehicle; ##$P<.01$, ###$P<.001$ vs Stress/Vehicle (2-way ANOVA with PLSD).

Table 3. Gene Expression Analysis of Interneuron Markers *Pvb*, *Sst*, *Calb*, and *Npy* in the Prefrontal Cortex of Rats Exposed to CMS

	No Stress	Lurasidone	Stress	Lurasidone/Stress
Pvb	100±5	92±6	95±5	90±3
Sst	100±6	117±8	103±5	110±8
Calb	100±5	99±8	97±4	99±3
Npy	100±4	96±3	96±6	97±4

The mRNA levels of parvalbumin (*Pvb*), somatostatin (*Sst*) neuropeptide y (*Npy*) and calbindin (*Calb*) were measured in the prefrontal cortex of rats exposed to CMS, in combination with chronic treatment with vehicle or lurasidone. The data expressed as a percentage of unstressed rats treated with vehicle (No Stress/Vehicle set at 100%) are the mean +/- SEM of at least 7 independent determinations.

Analysis of the NRF2-KEAP1 Antioxidant System in the Dorsal Hippocampus of Animals Exposed to CMS and Treated with Lurasidone

The NRF2 and the Kelch-like ECH-associated protein 1 (KEAP1) have a pivotal role in the control of the cellular antioxidant response. Indeed, upon nuclear translocation NRF2 binds to its consensus sequences (the so-called antioxidant responsive elements) to promote the transcription of several enzymes involved in the cellular mechanisms of detoxification. The transcription factor interacts in the cytosol with KEAP1, a chaperon protein that prevents its translocation into the nucleus, thus inhibiting its transcriptional antioxidant activity.

When considering the expression of the transcription factor *Nrf2*, we found a statistically significant interaction between CMS and lurasidone treatment ($F_{3,36}=11.616$, $P<.01$). Indeed, as shown in Figure 4A, while CMS exposure produced a slight, non-significant decrease of the transcription factor (-11% vs No Stress/Vehicle, $P>0.05$), lurasidone was able to increase its mRNA levels only when administered to CMS animals (+24% vs Stress/Vehicle, $P<.01$). Based on gene expression analyses, we decided to deepen our investigation by assessing the protein levels of NRF2 as well as of its inhibitor KEAP1 in the D-HIP.

The analysis of the protein levels of NRF2 revealed a significant stress*lurasidone interaction ($F_{3,32}=4.195$, $P<.05$). Indeed, as shown in Figure 4B, the protein levels of NRF2 were decreased by CMS (-40% vs No Stress/Vehicle, $P<.05$), an effect that was, at least in part, restored by lurasidone treatment considering that the levels of NRF2 protein in Stress/Lurasidone group did not differ from sham rats.

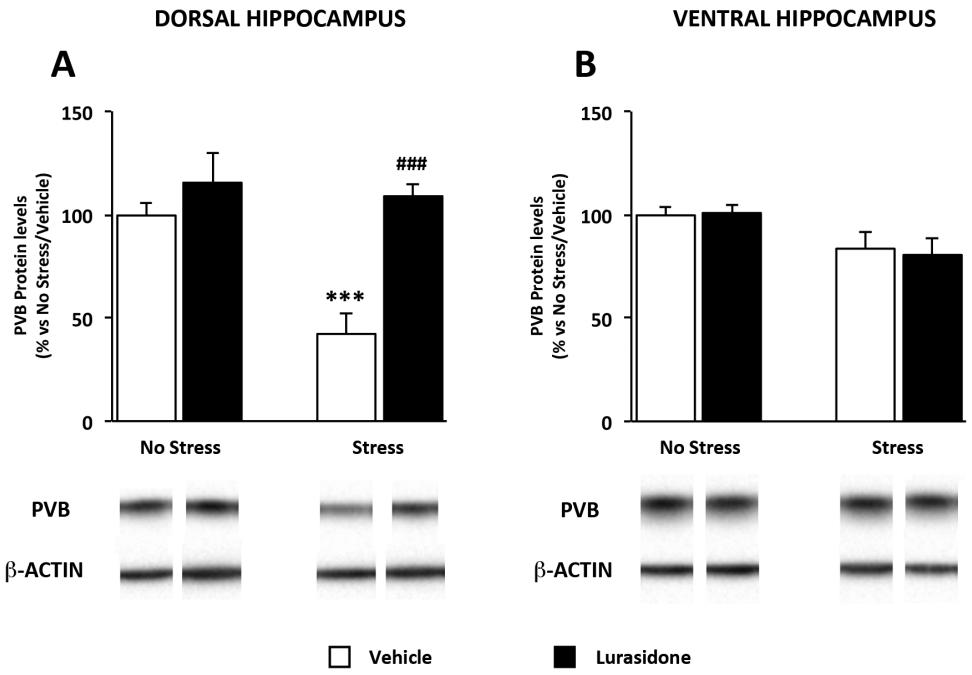

Figure 2. Protein expression of parvalbumin (PVB) in the hippocampus of rats exposed to chronic mild stress (CMS): modulation by lurasidone treatment. The protein levels of PVB were measured in the dorsal (A) and ventral (B) hippocampus of rats exposed to CMS in combination with chronic treatment with vehicle or lurasidone. The data, expressed as a percentage of unstressed rats treated with vehicle (No Stress/Vehicle, set at 100%), are the mean +/- SEM of at least 6 independent determinations. Representative western blot bands of PVB are shown under the respective graphs. ***P < .001 vs No Stress/Vehicle; ###P < .001 vs Stress/Vehicle (2-way ANOVA with PLSD).

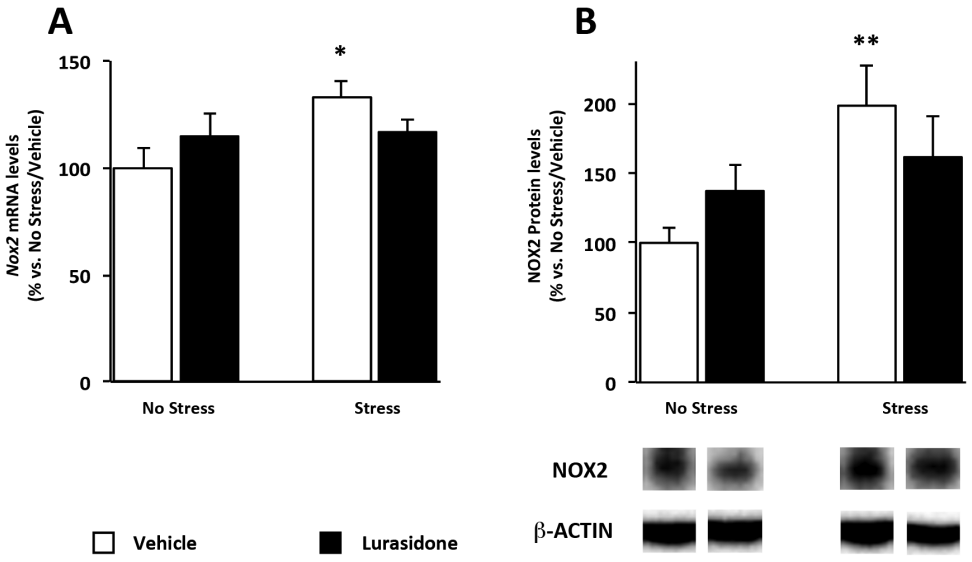

Figure 3. Gene expression and protein analyses of NADH oxidase-2 (NOX2) in the dorsal hippocampus of rats exposed to chronic mild stress (CMS): modulation by lurasidone treatment. The mRNA (A) and the protein levels (B) of NOX2 were measured in the dorsal hippocampus of rats exposed to CMS in combination with chronic treatment with vehicle or lurasidone. The data, expressed as a percentage of unstressed rats treated with vehicle (No Stress/Vehicle, set at 100%), are the mean +/- SEM of at least 8 independent determinations. Representative western-blot bands of NOX2 are shown under the respective graph. *P < .05, **P < .01 vs No Stress/Vehicle (2-way ANOVA with PLSD).

Interestingly, KEAP1 levels were strongly modulated by chronic lurasidone treatment ($F_{3,30}$=15.226, $P < .001$). Indeed, although CMS exposure did not affect KEAP1 levels, chronic lurasidone administration significantly reduced its protein levels in sham (-48% vs No Stress/Vehicle, $P < .01$; Figure 4C) as well as in CMS rats (-55% vs Stress/Vehicle, $P < .05$; Figure 4C).

Pearson Correlation Analysis between NRF2/KEAP1 Protein Levels and PVB in the Dorsal Hippocampus of Animals Exposed to CMS and Treated with Lurasidone

Next, to establish a potential relationship between the effects of stress exposure and pharmacological treatment on PVB

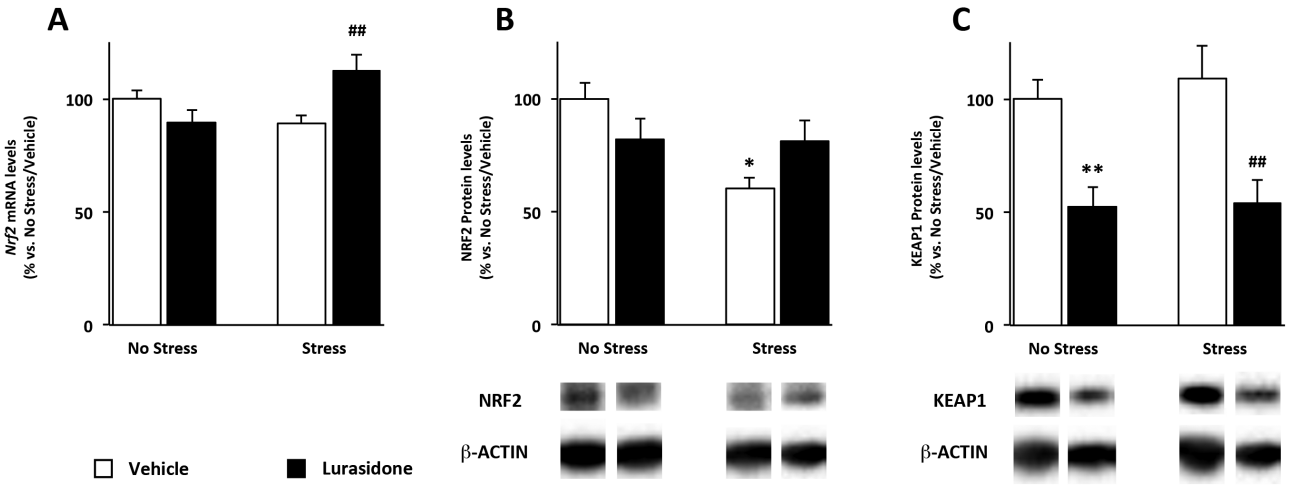

Figure 4. Analysis of nuclear factor-E2-related factor-2 (NRF2)-kelch like ECH associated protein-1 (KEAP1) expression in the dorsal hippocampus of rats exposed to chronic mild stress: modulation by lurasidone treatment. The mRNA (A) and protein levels (B) of NRF2 and the protein levels of the chaperon protein KEAP1 (C) were measured in the cytosolic fraction of the dorsal hippocampus of rats exposed to chronic mild stress (CMS) in combination with chronic treatment with vehicle or lurasidone. The data, expressed as a percentage of unstressed rats treated with vehicle (No Stress/Vehicle, set at 100%), are the mean +/- SEM of at least 7 independent determinations. Representative western-blot bands of NRF2 and KEAP1 are shown under the respective graphs. *$P<.05$, **$P<.01$ vs No Stress/Vehicle; ##$P<.01$ vs Stress/Vehicle (2-way ANOVA with PLSD).

expression with the levels of NRF2/KEAP1 antioxidant system, we performed a Pearson product-moment correlation coefficient analysis between the protein levels of NRF2 or KEAP1 and the protein levels of PVB in the D-HIP. As presented in Figure 5, NRF2 showed a significant positive correlation with the GABAergic marker ($r=0.414$, $P<.05$; Figure 5A), while a negative correlation was observed between the chaperone protein KEAP1 and PVB ($r=-0.501$, $P<.01$; Figure 5B).

Analysis of the Transcriptional Effects of NRF2 in the Dorsal Hippocampus of Animals Exposed to CMS and Treated with Lurasidone

Based on the changes in the functional interplay between NRF2 and KEAP1 after CMS exposure and/or lurasidone treatment, we decided to investigate the expression of some genes downstream from the transcriptional activity of NRF2, namely the enzymes *Srnx1*, *Ho-1*, *Nqo1*, and *Cat*.

As depicted in Figure 6, the expression of *Srxn1* (A) and *Ho-1* (B) were not modulated by CMS exposure or chronic lurasidone treatment. However, we found that the mRNA levels of *Nqo1* showed a significant stress x treatment interaction ($F_{3,37}=7.806$, $P<.01$). Indeed, chronic treatment with lurasidone was able to upregulate its expression, only when administered to CMS animals (+23% vs Stress/Vehicle, $P<.01$; Figure 6C). Moreover, we found a significant main effect of lurasidone treatment ($F_{3,38}=6.334$, $P<.05$) on *Cat* gene expression. Specifically, the pharmacological treatment increased the levels of the enzyme, but only when it was administered to sham animals (+21% vs No Stress/Vehicle, $P<.05$; Figure 6D).

Discussion

In recent years the interest in GABAergic interneurons has gained much attention due to their key role in functions that are altered in different psychiatric disorders, including major depression and schizophrenia (Luscher and Fuchs, 2015; Owen et al., 2016). Within this context, our data point out that the restorative effect of pharmacological treatment on PVB expression may be mediated by the drug-mediated regulation of the oxidative balance within the brain.

Most PVB-expressing cells are present in the central nervous system as interneurons, particularly within selected brain structures, including cerebral cortex, hippocampus, cerebellum, and spinal cord (Zaletel et al., 2016). In the hippocampus, a critical brain area involved in the control of emotional states, stress response, and cognitive function (Fanselow and Dong, 2010), PVB+ cells are mostly fast-spiking GABAergic interneurons that control the circuitry activity of pyramidal cells through their inhibitory activity. The reduction of PVB expression found in the D-HIP of CMS exposed rats is in line with the detrimental effects of stress or other adverse manipulations on the GABAergic system reported in other preclinical studies. For example, Czeh and collaborators have shown that 5 weeks of psychosocial stress were able to impair PVB expression in specific subregions of treeshrew hippocampus (Czeh et al., 2005). Similar results were obtained with other stress paradigms in rats, such as chronic immobilization (Hu et al., 2010) and social isolation (Filipović et al., 2013). Interestingly, in our experimental setting, the D-HIP appears to be more vulnerable to the impact of CMS, an effect that is in line with the results of Czeh and co-workers (Czéh et al., 2015). Considering that functional alterations of this hippocampal subregion have a major role in cognitive dysfunctions, we hypothesize that reduced PVB expression may contribute to the impaired cognitive function we have recently shown in rats exposed to the CMS paradigm (Calabrese et al., 2017). It is likely that the expression of PVB may be due to a decrease of protein expression instead of to cell loss. Indeed, as demonstrated by others, chronic stress exposure does not increase caspase-3 expression in GABAergic interneurons (Filipović et al., 2013). Interestingly, we found a strong induction of SST expression in the D-HIP following stress exposure. In both hippocampus and neocortex, PVB+ interneurons target the soma and the perisomatic dendrites of pyramidal neurons, controlling the output signaling of principal excitatory neurons. In parallel, SST interneurons generally target the more distal dendrites, gating the excitatory signals (Horn and Nicoll, 2018). Moreover, the activity of PVB

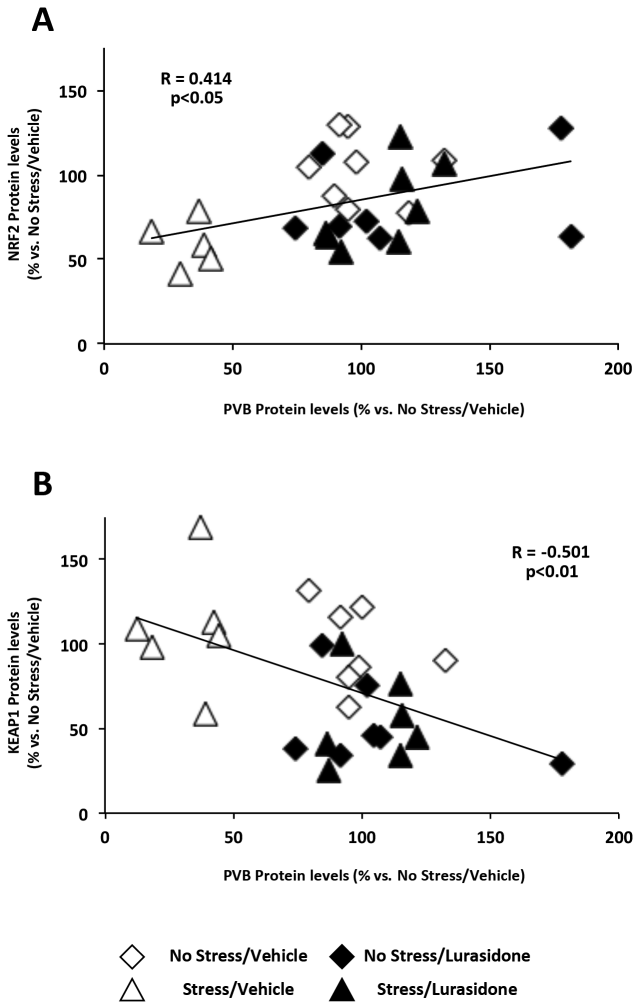

Figure 5. Pearson correlation analysis between nuclear factor-E2-related factor-2 (NRF2) or kelch like ECH associated protein-1 (KEAP1) and parvalbumin (PVB) protein levels in the dorsal hippocampus. The Pearson moment-product correlation (r) between NRF2 (A), KEAP1 (B), and PVB protein levels were measured in the dorsal hippocampus of rats exposed to chronic mild stress (CMS) in combination with chronic treatment with vehicle or lurasidone. The statistical significance was assumed with $P < .05$.

and SST interneurons is strictly interconnected as part of complex inhibitory microcircuits involved in the control of behavior and learning (Caroni, 2015). Considering that SST decrease has been causally related to anxiety/depression-like behaviors (Lin and Sibille, 2015), our result may seem counterintuitive. However, the increased expression of SST may represent a compensatory mechanism to limit the impaired somato-dendritic inhibition of pyramidal neurons after CMS-induced PVB loss. In parallel, the increase of SST observed after lurasidone treatment may be related to a specific mechanism induced by the drug, which is independent from the model analyzed. Indeed, we have previously shown a similar effect in serotonin transporter knockout rats treated with lurasidone (Luoni et al., 2013). In this sense, further functional studies are needed to better clarify the impact of pharmacological treatment with psychotropic drugs on these interneuron populations.

The opposite effects of CMS on these two markers may also explain why the protein levels of the glutamic acid decarboxylase 67 were not modulated by stress exposure in the D-HIP (data not shown). Indeed, we may speculate that in our experimental setting this enzyme is differentially modulated in the diverse interneuron subpopulations.

While the precise detrimental mechanisms triggered by stress exposure on PVB interneurons are not well clarified, the selective vulnerability of PVB+ interneurons to chronic stress may be due to their peculiar fast spiking activity. The firing of PVB interneurons requires a high amount of energy, and the increased metabolic activity under certain conditions, such as stress, may expose PVB neurons to potentially toxic effects of reactive oxygen and nitrogen species, which alter the redox balance of the cell (Kann, 2016).

In this respect, the NOX family represents a very important group of enzymes that, especially in the injured nervous system, is a major source of reactive oxygen species (Cooney et al., 2013). The upregulation of NOX2 expression after stress suggests an increase of the prooxidative activity in rats exposed to CMS. Increased levels of NOX2 have also been observed in brain areas of animals exposed to prolonged social isolation from weaning (Schiavone et al. 2009) as well as in a model of posttraumatic stress disorder, where NOX2 upregulation was paralleled by a decrease of PVB expression (Sun et al., 2016). Considering that NOX2 has been reported as the primary phagocytic oxidase (Bermudez et al., 2016), its altered expression may be associated with the increased activity of microglial cells under stressful conditions. In parallel, the production of prooxidative agents from NOX2 induces the activation of microglia, triggering a detrimental inflammatory loop, potentially harmful for neurons (Vilhardt et al., 2017). This hypothesis is supported by previous data from our laboratory showing increased expression of hippocampal CD11b, a marker of microglia activation, in animals exposed to 7 weeks of CMS (Rossetti et al., 2016). Although in the present study we measured the levels of gp190^{PHOX}, the principal membrane subunit of NOX2 enzyme, without evaluating other subunits of the enzymatic complex, we believe that the expression of the fundamental enzymatic subunit may reflect an increased response of the prooxidative system within the hippocampus. The detrimental effects of oxidative stress on PVB expression observed in response to CMS may also be the result of a glucocorticoid receptor (GR)-dependent extragenomic mechanism, which may affect the firing activity of these interneurons. Indeed, it has been proposed that the activation of membrane bound GR may induce the production of nitric oxide (NO), a small neurotransmitter responsible of the activation of PVB positive interneurons (Hu et al., 2010). The sustained activation of GR during chronic stressful condition may decrease PVB expression by sensitizing interneuron activation and, possibly, through a toxic effect due to NO release. Indeed, NO may be converted in different reactive nitrogen species, thus leading to oxidative damage of proteins, lipids, and DNA that are able to alter neuronal homeostasis (Moniczewski et al., 2015). We also showed that prolonged stress exposure alters the NRF2-KEAP-antioxidant responsive element system, a master regulator of the antioxidative response (Sandberg et al., 2014), with a decrease of NRF2 expression that may impair the activation of antioxidant mechanisms. Our results are in line with previous studies showing the negative effects of stress on NRF2. For example, a decreased expression of NRF2, with a parallel increase of oxidative stress, was found in social defeat-vulnerable animals exposed to chronic stress (Bouvier et al., 2017) as well as in rats exposed to 4 weeks of chronic stress (Omar and Tash, 2017).

Chronic treatment with lurasidone, an antipsychotic drug approved for the treatment of schizophrenia and bipolar depression, was able to normalize CMS-induced decrease of

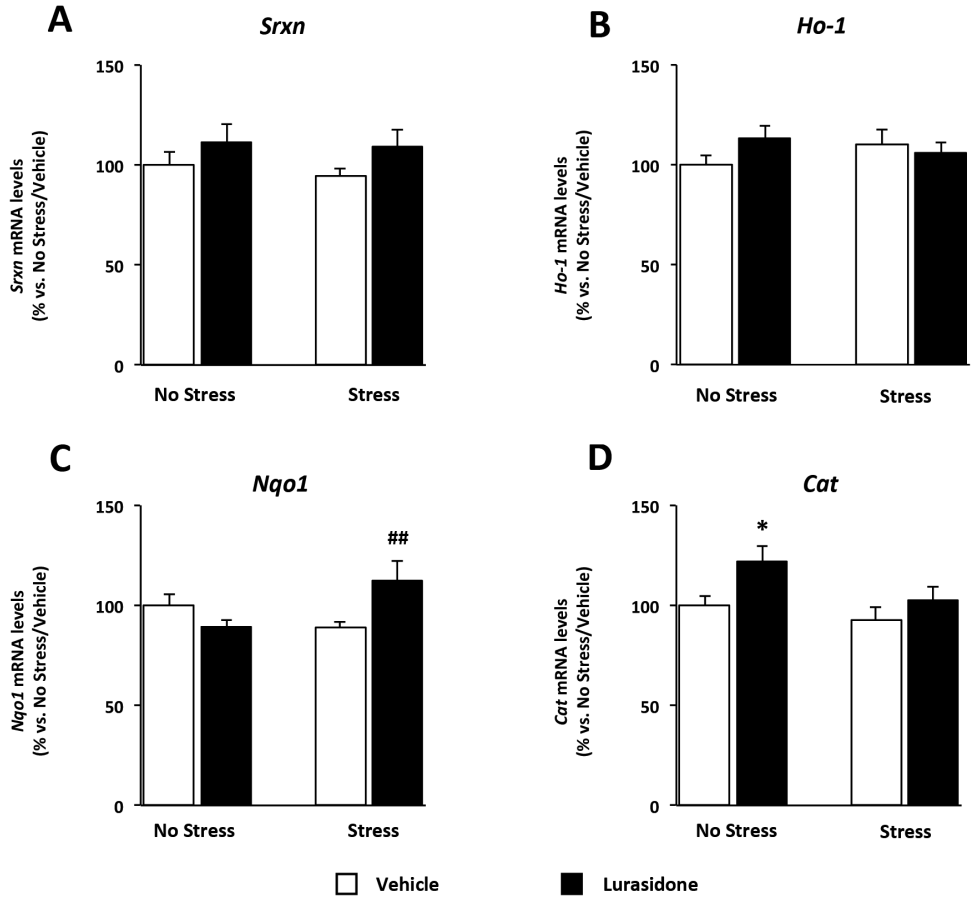

Figure 6. Analysis of nuclear factor-E2-related factor-2 (NRF2)-induced transcriptional response in the dorsal hippocampus of rats exposed to chronic mild stress (CMS): modulation by lurasidone treatment. The mRNA levels of Sufiredoxin (*Srxn1*, A), Heme oxigenase-1 (*Ho-1*, B), NAD(P)H dehydrogenase [quinone]-1 (*Nqo1*, C), and Catalase (*Cat*, D) were measured in the dorsal hippocampus of rats exposed to CMS in combination with chronic treatment with vehicle or lurasidone. The data, expressed as a percentage of unstressed rats treated with vehicle (No Stress/Vehicle, set at 100%), are the mean +/- SEM of at least 8 independent determinations. *$P < .01$ vs No Stress/Vehicle; ##$P < .01$ vs Stress/Vehicle (2-way ANOVA with PLSD).

PVB expression in D-HIP. In this sense, other studies support the idea that antipsychotic drugs, such as clozapine (Filipovic et al., 2017) and risperidone (Piontkewitz et al., 2012), may regulate the function of GABAergic interneurons through the modulation of specific markers. While these drugs share high affinity for 5-HT7 receptors, it is difficult to ascertain if this is the unique mechanism through which these agents modulate GABAergic function, considering the vast heterogeneity in their receptor profiles. Indeed, we believe that the observed effects represent adaptive mechanisms following prolonged drug administration regulating complex neuronal circuits that will eventually lead to changes in selective GABAergic subtypes. Lastly, the effect of lurasidone closely resembles what we have recently observed in the hippocampus of adult mice exposed to prenatal immune challenge (Luoni et al., 2017).

Due to the peculiar receptor profile of lurasidone, it is difficult to enlighten a molecular mechanism responsible for the effects of the pharmacological treatment on GABAergic interneurons. Our results, however, suggest that the ability of lurasidone to modulate the oxidative stress balance may be part of its restorative effect. Indeed, chronic lurasidone treatment was able to induce the gene expression of NRF2 only in stressed animals, suggesting an antioxidative effect of the pharmacological treatment only under adverse conditions. The positive effect of lurasidone was also found at the translational level, since the CMS-induced decrease of NRF2 was partially normalized in animals treated with the antipsychotic drug. Interestingly lurasidone is also able to regulate KEAP1, a chaperone protein that segregates NRF2 into the cytosol and promotes its proteasome-mediated degradation (Sandberg et al., 2014). Indeed, while KEAP1 protein levels in the cytosolic fraction were not altered by CMS exposure, we found that lurasidone treatment was able to reduce its levels, suggesting that the drug not only increases the expression of NRF2 but may also promote its activity through a negative modulation of KEAP1.

Our results suggest that, in addition to synaptic and neuroplastic mechanisms (Tarazi and Riva, 2013; Luoni et al., 2014), lurasidone is able to modulate the brain oxidative balance, which may contribute to its therapeutic effects and eventually enhance neuronal resiliency. Interestingly, similar mechanisms have also been described for other psychotropic drugs, including antidepressants (Martín-Hernández et al., 2016; Omar and Tash, 2017) and antipsychotics (MacDowell et al., 2016). It is interesting to note that the modulation of NRF2 and KEAP1 showed a significant Pearson correlation (positive and negative, respectively) with PVB protein levels, providing further support to the notion that the alterations of the GABAergic system following CMS exposure may be causally linked to a dysregulation of the oxidative balance in the D-HIP. In addition, the pharmacological treatment with lurasidone was able to induce the

expression of key antioxidant enzymes related to NRF2 transcriptional activity. The specific increased levels of *Nqo1* and *Catalase* support the idea of an antioxidative activity of the drug. This is in line with previously published data showing that, although stress exposure did not impair the transcription of antioxidant enzyme, the administration of an atypical antipsychotic increased antioxidant response in stressed animals (MacDowell et al., 2016).

In summary, the susceptibility of PVB neurons to stress may represent a key mechanism contributing to functional and structural deterioration in specific brain regions, such as the D-HIP, associated with psychiatric illness. The ability to counteract PVB alterations, for example with antioxidants/redox regulators (Steullet et al., 2017), or to promote the activity of PVB neurons (Chen et al., 2017) may represent a novel and important strategy to promote resilience. In this respect, our data provide new insights on the mechanism of action of lurasidone in the context of stress-related hippocampal dysfunction, suggesting that its pharmacological profile, which can improve neuronal/synaptic plasticity in hippocampus and cortex through both protective (antioxidant) and functional (BDNF) (Luoni et al., 2014) mechanisms, should support clinical efficacy reported in schizophrenia and bipolar disorder.

Acknowledgments

We thank Francesca Nirella for her scientific support for part of the work. We are grateful to Sumitomo Dainippon Pharma Co. Ltd for the generous gift of lurasidone.
This work was supported by the Italian Ministry of Instruction, University and Research (PRIN grant no. 2015SKN9YT to M.A.R.), Progetto Eccellenza, and Sumitomo Dainippon Pharma Co. Ltd. (to M.A.R.). Part of this work was supported by the statutory activity of the Institute of Pharmacology, Polish Academy of Sciences (Krakow, Poland) (to M.P.). All funding bodies had no role in designing the study.

Statement of Interest

M.A.R. received compensation as speaker/consultant from Lundbeck, Otzuka, Sumitomo Dainippon Pharma, and Sunovion, and he has received research grants from Lundbeck, Sumitomo Dainippon Pharma, and Sunovion. All other authors declare no financial interest or potential conflict of interest.

References

Begni V, Riva MA, Cattaneo A (2017) Cellular and molecular mechanisms of the brain-derived neurotrophic factor in physiological and pathological conditions. Clin Sci (Lond) 131:123–138.

Bermudez S, Khayrullina G, Zhao Y, Byrnes KR (2016) NADPH oxidase isoform expression is temporally regulated and may contribute to microglial/macrophage polarization after spinal cord injury. Mol Cell Neurosci 77:53–64.

Bouvier E, Brouillard F, Molet J, Claverie D, Cabungcal JH, Cresto N, Doligez N, Rivat C, Do KQ, Bernard C, Benoliel JJ, Becker C (2017) Nrf2-dependent persistent oxidative stress results in stress-induced vulnerability to depression. Mol Psychiatry 22:1795.

Calabrese F, Riva MA, Molteni R (2016a) Synaptic alterations associated with depression and schizophrenia: potential as a therapeutic target. Expert Opin Ther Targets 20:1195–1207.

Calabrese F, Savino E, Papp M, Molteni R, Riva MA (2016b) Chronic mild stress-induced alterations of clock gene expression in rat prefrontal cortex: modulatory effects of prolonged lurasidone treatment. Pharmacol Res 104:140–150.

Calabrese F, Brivio P, Gruca P, Lason-Tyburkiewicz M, Papp M, Riva MA (2017) Chronic mild stress-induced alterations of local protein synthesis: a role for cognitive impairment. ACS Chem Neurosci 8:817–825.

Caroni P (2015) Inhibitory microcircuit modules in hippocampal learning. Curr Opin Neurobiol 35:66–73.

Cattaneo A, Riva MA (2016) Stress-induced mechanisms in mental illness: a role for glucocorticoid signalling. J Steroid Biochem Mol Biol 160:169–174.

Chen CC, Lu J, Yang R, Ding JB, Zuo Y (2017) Selective activation of parvalbumin interneurons prevents stress-induced synapse loss and perceptual defects. Mol Psychiatry doi: 10.1038/mp.2017.159.

Cooney SJ, Bermudez-Sabogal SL, Byrnes KR (2013) Cellular and temporal expression of NADPH oxidase (NOX) isotypes after brain injury. J Neuroinflammation 10:155.

Czeh B, Simon M, van der Hart MG, Schmelting B, Hesselink MB, Fuchs E (2005) Chronic stress decreases the number of parvalbumin-immunoreactive interneurons in the hippocampus: prevention by treatment with a substance P receptor (NK1) antagonist. Neuropsychopharmacology 30:67–79.

Czéh B, Varga ZK, Henningsen K, Kovács GL, Miseta A, Wiborg O (2015) Chronic stress reduces the number of gabaergic interneurons in the adult rat hippocampus, dorsal-ventral and region-specific differences. Hippocampus 25:393–405.

Fanselow MS, Dong HW (2010) Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65:7–19.

Filipović D, Zlatković J, Gass P, Inta D (2013) The differential effects of acute vs Chronic stress and their combination on hippocampal parvalbumin and inducible heat shock protein 70 expression. Neuroscience 236:47–54.

Filipović D, Stanisavljević A, Jasnić N, Bernardi RE, Inta D, Perić I, Gass P (2017)
Chronic treatment with fluoxetine or clozapine of socially isolated rats prevents subsector-specific reduction of parvalbumin immunoreactive cells in the hippocampus. Neuroscience 371:384–394.

Freund TF (2003) Interneuron diversity series: rhythm and mood in perisomatic inhibition. Trends Neurosci 26:489–495.

Harvey PD, Siu CO, Hsu J, Cucchiaro J, Maruff P, Loebel A (2013) Effect of lurasidone on neurocognitive performance in patients with schizophrenia: a short-term placebo- and active-controlled study followed by a 6-month double-blind extension. Eur Neuropsychopharmacol 23:1373–1382.

Horn ME, Nicoll RA (2018) Somatostatin and parvalbumin inhibitory synapses onto hippocampal pyramidal neurons are regulated by distinct mechanisms. Proc Natl Acad Sci U S A 115:589–594.

Hu W, Zhang M, Czéh B, Flügge G, Zhang W (2010) Stress impairs gabaergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. Neuropsychopharmacology 35:1693–1707.

Kann O (2016) The interneuron energy hypothesis: implications for brain disease. Neurobiol Dis 90:75–85.

Kupfer DJ, Frank E, Phillips ML (2012) Major depressive disorder: new clinical, neurobiological, and treatment perspectives. Lancet 379:1045–1055.

Lin LC, Sibille E (2015) Somatostatin, neuronal vulnerability and behavioral emotionality. Mol Psychiatry 20:377–387.

Loebel A, Cucchiaro J, Silva R, Kroger H, Hsu J, Sarma K, Sachs G (2014) Lurasidone monotherapy in the treatment of bipolar I depression: a randomized, double-blind, placebo-controlled study. Am J Psychiatry 171:160–168.

Luoni A, Hulsken S, Cazzaniga G, Racagni G, Homberg JR, Riva MA (2013) Behavioural and neuroplastic properties of chronic lurasidone treatment in serotonin transporter knockout rats. Int J Neuropsychopharmacol 16:1319–1330.

Luoni A, Macchi F, Papp M, Molteni R, Riva MA (2014) Lurasidone exerts antidepressant properties in the chronic mild stress model through the regulation of synaptic and neuroplastic mechanisms in the rat prefrontal cortex. Int J Neuropsychopharmacol 18. doi: 10.1093/ijnp/pyu061.

Luoni A, Richetto J, Longo L, Riva MA (2017) Chronic lurasidone treatment normalizes gabaergic marker alterations in the dorsal hippocampus of mice exposed to prenatal immune activation. Eur Neuropsychopharmacol 27:170–179.

Luscher B, Fuchs T (2015) Gabaergic control of depression-related brain states. Adv Pharmacol 73:97–144.

MacDowell KS, Caso JR, Martín-Hernández D, Moreno BM, Madrigal JLM, Micó JA, Leza JC, García-Bueno B (2016) The atypical antipsychotic paliperidone regulates endogenous antioxidant/anti-inflammatory pathways in rat models of acute and chronic restraint stress. Neurotherapeutics 13:833–843.

Martín-Hernández D, Bris ÁG, MacDowell KS, García-Bueno B, Madrigal JL, Leza JC, Caso JR (2016) Modulation of the antioxidant nuclear factor (erythroid 2-derived)-like 2 pathway by antidepressants in rats. Neuropharmacology 103:79–91.

Millan MJ, et al. (2012) Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. Nat Rev Drug Discov 11:141–168.

Molteni R, Rossetti AC, Savino E, Racagni G, Calabrese F (2016) Chronic mild stress modulates activity-dependent transcription of BDNF in rat hippocampal slices. Neural Plast 2016:2592319.

Moniczewski A, Gawlik M, Smaga I, Niedzielska E, Krzek J, Przegaliński E, Pera J, Filip M (2015) Oxidative stress as an etiological factor and a potential treatment target of psychiatric disorders. Part 1. Chemical aspects and biological sources of oxidative stress in the brain. Pharmacol Rep 67:560–568.

Nasrallah HA, Cucchiaro JB, Mao Y, Pikalov AA, Loebel AD (2015) Lurasidone for the treatment of depressive symptoms in schizophrenia: analysis of 4 pooled, 6-week, placebo-controlled studies. CNS Spectr 20:140–147.

Omar NN, Tash RF (2017) Fluoxetine coupled with zinc in a chronic mild stress model of depression: providing a reservoir for optimum zinc signaling and neuronal remodeling. Pharmacol Biochem Behav 160:30–38.

Owen MJ, Sawa A, Mortensen PB (2016) Schizophrenia. Lancet 388:86–97.

Perova Z, Delevich K, Li B (2015) Depression of excitatory synapses onto parvalbumin interneurons in the medial prefrontal cortex in susceptibility to stress. J Neurosci 35:3201–3206.

Piontkewitz Y, Bernstein HG, Dobrowolny H, Bogerts B, Weiner I, Keilhoff G (2012) Effects of risperidone treatment in adolescence on hippocampal neurogenesis, parvalbumin expression, and vascularization following prenatal immune activation in rats. Brain Behav Immun 26:353–363.

Pittenger C, Duman RS (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 33:88–109.

Rossetti AC, Papp M, Gruca P, Paladini MS, Racagni G, Riva MA, Molteni R (2016) Stress-induced anhedonia is associated with the activation of the inflammatory system in the rat brain: restorative effect of pharmacological intervention. Pharmacol Res 103:1–12.

Sandberg M, Patil J, D'Angelo B, Weber SG, Mallard C (2014) NRF2-regulation in brain health and disease: implication of cerebral inflammation. Neuropharmacology 79:298–306.

Schiavone S, Sorce S, Dubois-Dauphin M, Jaquet V, Colaianna M, Zotti M, Cuomo V, Trabace L, Krause KH (2009) Involvement of NOX2 in the development of behavioral and pathologic alterations in isolated rats. Biol Psychiatry 66:384–392.

Smaga I, Niedzielska E, Gawlik M, Moniczewski A, Krzek J, Przegaliński E, Pera J, Filip M (2015) Oxidative stress as an etiological factor and a potential treatment target of psychiatric disorders. Part 2. Depression, anxiety, schizophrenia and autism. Pharmacol Rep 67:569–580.

Steullet P, Cabungcal JH, Coyle J, Didriksen M, Gill K, Grace AA, Hensch TK, LaMantia AS, Lindemann L, Maynard TM, Meyer U, Morishita H, O'Donnell P, Puhl M, Cuenod M, Do KQ (2017) Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. Mol Psychiatry 22:936–943.

Sun XR, Zhang H, Zhao HT, Ji MH, Li HH, Wu J, Li KY, Yang JJ (2016) Amelioration of oxidative stress-induced phenotype loss of parvalbumin interneurons might contribute to the beneficial effects of environmental enrichment in a rat model of post-traumatic stress disorder. Behav Brain Res 312:84–92.

Tarazi FI, Riva MA (2013) The preclinical profile of lurasidone: clinical relevance for the treatment of schizophrenia. Expert Opin Drug Discov 8:1297–1307.

Vilhardt F, Haslund-Vinding J, Jaquet V, McBean G (2017) Microglia antioxidant systems and redox signalling. Br J Pharmacol 174:1719–1732.

Willner P (2017) The chronic mild stress (CMS) model of depression: history, evaluation and usage. Neurobiol Stress 6:78–93.

Yatham LN, Mackala S, Basivireddy J, Ahn S, Walji N, Hu C, Lam RW, Torres IJ (2017) Lurasidone versus treatment as usual for cognitive impairment in euthymic patients with bipolar I disorder: a randomised, open-label, pilot study. Lancet Psychiatry 4:208–217.

Zaletel I, Filipović D, Puškaš N (2016) Chronic stress, hippocampus and parvalbumin-positive interneurons: what do we know so far? Rev Neurosci 27:397–409.