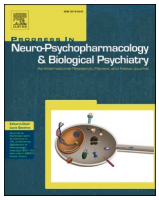
Progress in Neuropsychopharmacology & Biological Psychiatry 129 (2024) 110885

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp



Transcriptomic analyses of rats exposed to chronic mild stress: Modulation by chronic treatment with the antipsychotic drug lurasidone

Veronica Begni [a], Moira Marizzoni [b,c], Kerstin Camile Creutzberg [a], Diana Morena Silipo [a], Mariusz Papp [d], Annamaria Cattaneo [a,b], Marco Andrea Riva [a,b,*]

[a] Department of Pharmacological and Biomolecular Sciences, University of Milan, Via Balzaretti 9, 20133 Milan, Italy
[b] Biological Psychiatry Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Via Pilastroni 4, 25125 Brescia, Italy
[c] Lab of Neuroimaging and Alzheimer's Epidemiology, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Via Pilastroni 4, 25125 Brescia, Italy
[d] Institute of Pharmacology and Polish Academy of Sciences, Smętna Street 12, Kraków 31-343, Poland

ARTICLE INFO

Keywords:
Chronic mild stress
Anhedonia
Rats
Lurasidone
Transcriptomics
Prefrontal cortex

ABSTRACT

Exposure to stressful experiences accounts for almost half of the risk for mental disorders. Hence, stress-induced alterations represent a key target for pharmacological interventions aimed at restoring brain function in affected individuals. We have previously demonstrated that lurasidone, a multi-receptor antipsychotic drug approved for the treatment of schizophrenia and bipolar depression, can normalize the functional and molecular impairments induced by stress exposure, representing a valuable tool for the treatment of stress-induced mental illnesses. However, the mechanisms that may contribute to the therapeutic effects of lurasidone are still poorly understood. Here, we performed a transcriptomic analysis on the prefrontal cortex (PFC) of adult male rats exposed to the chronic mild stress (CMS) paradigm and we investigated the impact of chronic lurasidone treatment on such changes. We found that CMS exposure leads to an anhedonic phenotype associated with a down-regulation of different pathways associated to neuronal guidance and synaptic plasticity within the PFC. Interestingly, a significant part of these alterations (around 25%) were counteracted by lurasidone treatment. In summary, we provided new insights on the transcriptional changes relevant for the therapeutic intervention with lurasidone, which may ultimately promote resilience.

1. Introduction

Major depressive disorder is a chronic mental disease affecting approximately 350 million people around the world, representing a leading cause of disability and a major contributor to the overall global burden of disease (World Health Organization, 2022). The aetiology of depression is complex and comprises both genetic and environmental factors, such as the exposure to stress (Schmitt et al., 2014). While pharmacological treatments initially act at synaptic level, they may eventually lead to transcriptional changes contributing to functional and structural modifications that are essential for the therapeutic efficacy (Malhi and Mann, 2018). Several studies have investigated drug-induced gene expression alterations aimed at identifying relevant mechanisms of actions, although a deeper understating is still needed (Zygmunt et al., 2019). Whole-genome transcriptional profiling of antidepressant drugs as ketamine and imipramine revealed that ketamine is more active in the hippocampus (HIP) while imipramine's action mostly involves the nucleus accumbens (NAc) and amygdala (AMY) (Bagot et al., 2017). Furthermore, both ketamine and imipramine have been found capable to revert stress-induced alterations in the prefrontal cortex (PFC) of animals exposed to the chronic social defeat stress, an ethologically validated model of depression (Bagot et al., 2017). A transcriptome profiling of paroxetine effects in hippocampal dentate gyrus identified candidate mechanisms associated with antidepressant response, e.g., neuropeptide signalling, synaptic transmission, calcium signalling, and regulation of glucocorticoid secretion (Herzog et al., 2021). Similarly, a single dose of ketamine has been demonstrated to modulate calcium signalling, synaptic function and plasticity in glutamatergic neurons of the ventral HIP (Lopez et al., 2022). On the contrary, genome-wide gene expression analysis in peripheral blood distinguished very few differentially expressed genes related to antidepressant treatment with vortioxetine in patients with depression (Nøhr et al., 2021).

Lurasidone is an atypical antipsychotic agent, approved for the

* Corresponding author at: Department of Pharmacological and Biomolecular Sciences, University of Milan, Via Balzaretti 9, 20133 Milan, Italy.
E-mail address: m.riva@unimi.it (M.A. Riva).

https://doi.org/10.1016/j.pnpbp.2023.110885
Received 4 July 2023; Received in revised form 9 October 2023; Accepted 18 October 2023
Available online 20 October 2023

treatment of schizophrenia and bipolar depression, which, in addition to its antagonism at dopamine D2 receptors, is a potent antagonist at serotonin 5-HT2A and 5-HT7 receptors, and a partial agonist of serotonin 5-HT1A receptors (Tarazi and Riva, 2013). Even if lurasidone was initially introduced as an antipsychotic drug, there is growing evidence reporting its potential therapeutical properties to treat depressive-like phenotypes (Ali et al., 2020; DelBello et al., 2017). Accordingly, we have previously demonstrated that chronic lurasidone administration is able to normalize anhedonia and cognitive deficit induced by stress exposure (Luoni et al., 2015; Rossetti et al., 2018; Calabrese et al., 2020), as well as the impairments of coping abilities under challenging situations (Begni et al., 2022). At the molecular level, although we have demonstrated its ability in modulating several processes including the hypothalamic–pituitary–adrenal (HPA) axis, neuroplasticity, excitatory/inhibitory and oxidative mechanisms as well as neuroinflammation (de Bartolomeis et al., 2022; Sanson and Riva, 2020), a global transcriptomic analysis of the changes produced by chronic lurasidone treatment under stressful conditions has not been reported yet.

On these bases in the present work, we investigated the transcriptomic profiles of the PFC from rats exposed to chronic mild stress (CMS) which were chronically treated with lurasidone. The CMS depression model is considered as the animal model with the highest validity and translational potential to study depression (Willner, 2017). We focused on the PFC as one of the key brain areas implicated in the pathogenesis of emotional and cognitive symptoms of depression (P. Xu et al., 2019). Furthermore, as previously described, other antidepressant treatments showed their major therapeutic potential within this brain area, promoting resilience-related molecular adaptations (Bagot et al., 2017). In detail, we used RNA-sequencing to characterize the pathways and biological functions modulated by stress exposure and the potential of lurasidone in reverting such changes.

2. Methods

2.1. Experimental design and animals

Male Wistar rats ($n = 40$) were purchased from Charles River (Germany) and were delivered to the animal facility one month before the beginning of the experiment. Animals were first randomly divided into two groups, control (CT) and chronic mild stress (CMS). The experiment had a total duration of seven weeks and during the whole period, weekly sucrose intake measurements have been performed. During the first two weeks, animals from the CMS group were exposed to the stress protocol while CT animals were left undisturbed, except for cage cleaning and sucrose measures. From week three each group was further divided into two, resulting in four groups ($n = 10$ for each group): CT with vehicle treatment (CT VEH), CT with lurasidone treatment (CT LUR), CMS with vehicle treatment (CMS VEH), and CMS with lurasidone treatment (CMS LUR). Fig. 1 displays the experimental design (created with BioRender.com).

Animals were single-housed and had access to food and water ad libitum except during some stages of the experiment, as described below. Moreover, cages were maintained on a 12-h light/dark cycle (lights on at 08 am) in an environment with controlled temperature (22 ± 2 °C) and humidity (50 ± 5%) conditions. All procedures included in this study are in conformity with the rules and principles of the 86/609/EEC Directive and have been approved by the Local Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

2.2. Sucrose intake and stress protocol

After 3 weeks of laboratory and housing conditions habituation, the animals were trained to consume 1% sucrose solution, as previously reported (Begni et al., 2022). The training consisted of eight 1 h baseline tests, in which sucrose was presented, in the home cage, following 14 h of food and water deprivation. Sucrose bottles were weighed and placed in the home cage for one hour, after this period bottles were re-weighed, and sucrose intake was calculated. Subsequently, sucrose intake was measured weekly throughout the whole experiment, at the end of every week.

Animals were divided into two matched groups (CT and CMS). Animals in the CMS group were exposed to a chronic mild stress protocol for seven consecutive weeks (Begni et al., 2022). Each week of the stress protocol was composed by the following: two periods of food or water deprivation, two periods of 45-degree cage tilt, two periods of intermittent illumination (lights on and off every 2 h), two periods of soiled cage (250 ml water in sawdust bedding), one period of paired housing, two periods of low-intensity stroboscopic illumination (150 flashes/min), and three periods of no stress. Every period had a duration of 10 to 14 h without interruptions (day and night), and the sequence of stressors was different every week to avoid habituation. Control animals were housed in separate rooms and had no contact with stressed animals. The CT group was only deprived of food and water for 14 h preceding each sucrose test, but otherwise, food and water were freely available in the home cage as mentioned above.

2.3. Drug administration

After two weeks of stress exposure, CT and CMS animals were further divided into matched subgroups. For the next five weeks, animals received one oral daily administration of vehicle (1% (*w/v*) hydroxyethylcellulose) or lurasidone (3 mg/kg), per gavage. The volume of all administrations was set at 1 ml/kg. Drug administration occurred around 10 am and the weekly sucrose intake test was carried out 24 h after drug administration. The dose and administration route of lurasidone were selected based on previous studies demonstrating its antidepressant efficacy (Creutzberg et al., 2023; Luoni et al., 2015).

2.4. Sacrifice and brain sampling

The animals were decapitated 24 h after the last drug administration,

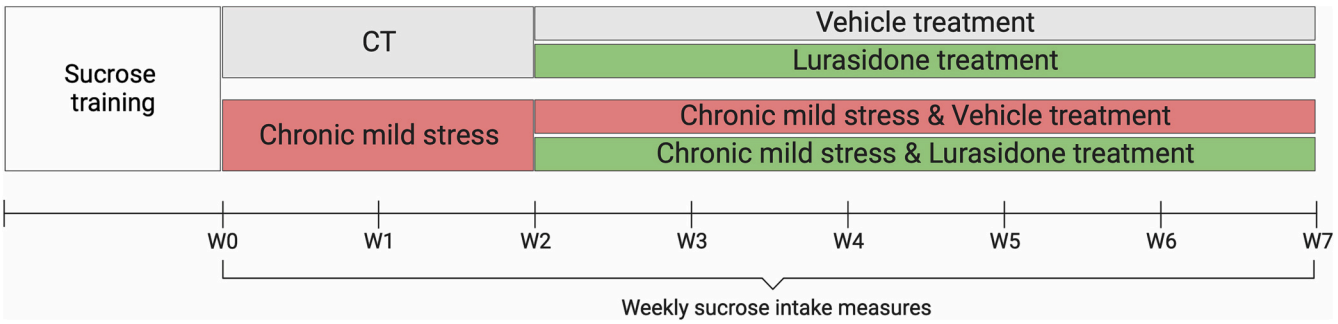


Fig. 1. Timeline of the experimental design. Created with BioRender.com.

the brain was extracted from the skull and placed on an ice-chilled plate. The prefrontal cortex was free-hand dissected and snap-frozen using dry ice and stored at −80 °C until RNA extraction.

2.5. RNA extraction and transcriptional analysis

RNA extraction was performed using PureZol RNA isolation reagent (Bio-Rad Laboratories, Italy) standard protocol, following manufacturers' instructions. RNA concentration was measured with NanoDrop spectrophotometer (ThermoFisher) while RNA quality was analyzed by using the TapeStation 4200 instrument (Agilent Technologies). We reported RNA Integrity Number (RIN) >8 for all the samples.

Transcriptome library preparation was conducted using the Illumina Stranded mRNA Prep Ligation kit. 6 samples per group (CT VEH, CMS VEH and CMS LUR) were used for library preparation and sequencing. Libraries were sequenced on a NextSeq550 platform (Illumina) by using High Output Kit v2.5 (150 Cycles) paired-ended, read length 74.

2.6. Data analysis and statistics

The data of sucrose intake were analyzed (GraphPad Prism 9) by analyses of variance (ANOVA) with three between-subject factors (CMS condition, drug treatment and successive sucrose tests). The Tukey's test comparison was used for post-hoc comparisons of means.

Regarding the RNAseq, the quality of the data was checked by using FastQ and the raw read counts were quantified at the transcript level using Salmon (v 1.4.0). Next, the transcript-level differential expression was assessed using DESeq2 (v1.30.1) in R. Filtering low-abundance data has been conducted and genes with less than ten reads across all samples have been filtered (Stupnikov et al., 2021). Differentially expressed genes were identified by applying a FC cut-off of 1.1, and an unadjusted p-value <0.05. Benjamini-Hochberg adjusted false discovery rate (FDR) was also used with a cut-off of 0.1 (q-value). Gene ontology analyses were performed using Ingenuity Pathway Analysis (IPA – QIAGEN Bioinformatics) software with p value <0.05. We used the z-score algorithm to identify pathways and biological functions that were expected to be activated or inhibited in our datasets. Z-scores >2 or < −2 indicate significantly modulated pathways and biological functions. Venn Diagrams were produced using BioVenn (Hulsen et al., 2008). Heatmaps were generated using Morpheus (Morpheus, https://software.broadinstitute.org/Morpheus). STRING app in Cytoscape Software (version 3.9.1) was used to detect the interactions among the DEGs. The networks consisted of one large, connected component, several smaller networks, and some unconnected nodes. We used only the largest connected components to run further analyses. Finally, the Gene Ontology Biological Processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the DEGs in the network were performed with the STRING Enrichment app in Cytoscape Software.

3. Results

3.1. Lurasidone normalizes CMS-induced anhedonia in rats

In accordance with our previous studies (Begni et al., 2022; Creutzberg et al., 2023; Luoni et al., 2015), exposure to CMS caused a decrease in the consumption of a sucrose solution, as a measure of anhedonia, which was normalized by sub-chronic lurasidone treatment (Interaction effect: $F(7, 888) = 3.650$; $p = 0.0007$). In fact, already after the first week of stress exposure, the intakes fell by approximately 37% in stressed animals ($p < 0.0001$). After 2 weeks of stress exposure, when sucrose intake was reduced by 46% ($p < 0.0001$), animals were randomized to receive vehicle of lurasidone. As shown in Fig. 2, while CMS rats treated with vehicle maintained the anhedonic phenotype ($p < 0.0001$), lurasidone administration in CMS rats produced a gradual improvement of the anhedonic phenotype, reaching statistical significance after three weeks of drug treatment ($p < 0.0001$). As compared to vehicle-treated group, five weeks of lurasidone treatment had no significant effect on the body weight of control and CMS animals (data not shown).

3.2. Neuronal cell death and viability in the PFC are key biological functions affected by CMS exposure

The PFC is one of the most sensitive areas to the effects of prolonged exposure to stress (Arnsten, 2009) and we previously shown that lurasidone can restore the deficits of synaptic plasticity as well as several molecular alterations in the PFC of rats exposed to CMS (Luoni et al., 2015). In order to gain further insight into these alterations, we applied a genome-wide approach in order to evaluate the transcriptomic alterations produced by CMS exposure and their modulation following lurasidone treatment.

We found 525 differentially expressed genes (DEGs) due to CMS exposure in the PFC of adult male animals, as compared to CT animals. 540 genes were instead differentially expressed within the PFC of CMS animals treated with lurasidone, as compared to vehicle-treated CMS animals. Almost half of DEGs in CMS animals compared to CT were downregulated (46.66%), while almost 70% of genes were upregulated

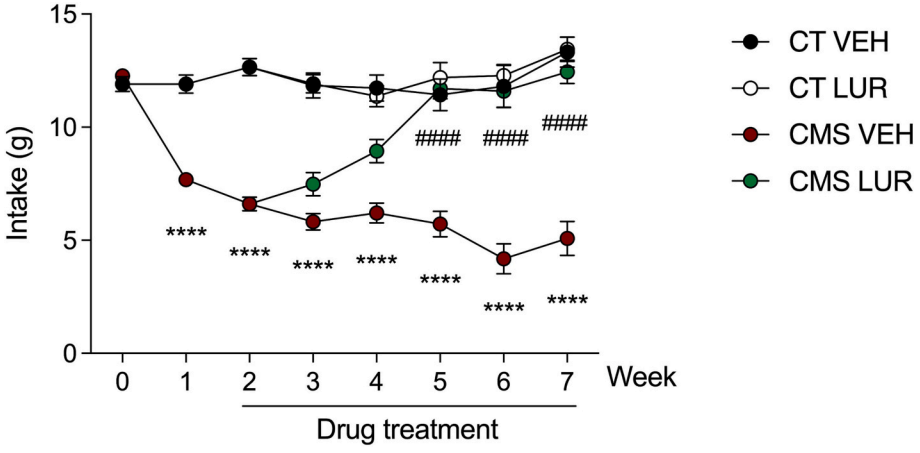


Fig. 2. Analysis of sucrose intake after CMS exposure and modulation by chronic lurasidone treatment.
Sucrose intake was measured at weekly intervals (at baseline, after 7 and 14 days of stress exposure and after 7, 14, 21, 28 and 35 days of drug treatment) in control (CT) or stressed (CMS) animals treated with vehicle (VEH) or lurasidone (LUR). Results are expressed as mean ± SEM of 10 animals per group. **** $p < 0.0001$ vs CT VEH; #### $p < 0.0001$ vs CMS VEH (Repeated measures ANOVA followed by post hoc tests).

by lurasidone treatment (66.11%). The volcano plot of DEGs and the percentage of up and downregulated genes for each comparison are displayed in Fig. 3 (A, B). Following the application of FDR correction (<0.1), expression changes due to CMS exposure reached significance in 4 genes, namely patatin-like phospholipase domain containing 2 (*Pnpla2*), retinol saturase (*Retsat*), ribosomal protein S6 (*Rps6*), and spermatogenesis and centriole associated 1-like (*Spatc1l*). *Pnpla2* was instead the only gene showing a differential expression when considering the effect of lurasidone treatment. Interestingly, CMS exposure produced a strong downregulation of *Pnpla2* mRNA levels (logFC = −28.970), while lurasidone treatment reverted such alteration (logFC = 19.189). *Retsat* (logFC = 0.943) and *Spatc1l* (logFC = 6.148) mRNA levels were significantly upregulated after CMS exposure while a significant downregulation was observed for *Rps6* (logFC = −10.165).

We then conducted a pathway analysis revealing that the 525 genes modulated by CMS exposure were enriched in 45 pathways (Supplementary Table 1). Filtering for nervous system-related pathways, we could identify 4 pathways, although none of these had $|z| > 2$ (Fig. 3C). One pathway, namely Regulation of Actin-based Motility by Rho, was upregulated (z-score = 1.134) while one was downregulated (GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells) (z-score = −0.333), and two pathways had unavailable z-score data. The genes belonging to the forementioned pathways are listed in Supplementary Table 1.

On the other hand, the pathway analysis revealed that the 540 genes modulated by lurasidone treatment in CMS rats were enriched in 44 pathways, which were mainly upregulated (Supplementary Table 2). Filtering for nervous system-related pathways, we could identify 15 pathways (Fig. 3D). >70% of these pathways were upregulated (11), while 1 pathway had a z-score equal to zero, 1 pathway was downregulated, and 2 pathways did not have available z-scores. Among the 11 upregulated pathways, 4 had z-scores>2, namely Gustation (z-score = 2.53), CREB (z-score = 2.6), Netrin (z-score = 2.646) and Opioid (z-score = 2.673) Signalling pathways. Furthermore, the analysis revealed the upregulation of Endocannabinoid Neuronal Synapse (z-score = 1.897), GNRH Signalling (z-score = 1.342), Neuropathic Pain Signalling In Dorsal Horn Neurons (z-score = 1.134), GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells (z-score = 1), Cholecystokinin/Gastrin-mediated Signalling (z-score = 0.816), Oxytocin Signalling (z-score = 0.577), and Dopamine-DARPP32 Feedback in cAMP Signalling (z-score = 0.447). Conversely, a downregulation of Neurovascular Coupling Signalling Pathway was observed after lurasidone treatment in CMS animals (z-score = −0.894). The genes belonging to the forementioned pathways are listed in Supplementary Table 2.

Next, we used IPA software to predict the possible modulation of downstream biological functions as a consequence of CMS exposure or lurasidone treatment. The analysis of biological functions of the 525 genes modulated by CMS exposure reported 6 significantly impacted biological functions, predicted to be mainly activated in CMS animals (Supplementary Table 3). These annotations comprised abnormality of cerebral cortex, neuronal cell death and brain damage (Fig. 3E). The only downregulated disease and function was the proliferation of neural cells, although with a minimal modulation (z-score = −0.093). The list of genes belonging to the forementioned biological functions is reported in Supplementary Table 3.

The analysis of biological functions of the 540 genes modulated by lurasidone treatment in CMS rats reported 13 significantly altered biological functions, predicted to be mainly activated in response to lurasidone treatment (Supplementary Table 4). These annotations mostly comprised cell viability although none of them displayed a $|z| > 2$ (Fig. 3F). Specifically, we found a prediction of activation for Synthesis of lipid, Proliferation of neural cells, Growth of neurites, Microtubule dynamics, Cell viability of cortical neurons, Cell viability of neurons, Cell viability of brain cells, Dendritic growth/branching, Branching of neurons, Neuronal cell death. On the contrary, Cell death of cerebral cortex cells, Cell death of cortical neurons and Neuritogenesis are predicted to be inhibited by lurasidone treatment within the PFC. The list of genes belonging to the forementioned biological functions is reported in Supplementary Table 4.

We next performed a network analysis on the 525 genes modulated by CMS (Supplementary Fig. S1). The network analysis highlighted a statistically significant association among the DEGs ($p = 1 \times 10^{-16}$), with 393 nodes and 876 edges (expected number of edges: 544). Enrichment analysis revealed the KEGG pathways potentially involved, including MAPK signalling (FDR = 0.0016), Ras signalling (FDR = 0.002), metabolic (FDR = 0.0037), leukocyte transendothelial migration (FDR = 0.0037) and Rap1 signalling (FDR = 0.0041) pathways. Furthermore, the network was significantly enriched for several GO biological processes, including regulation of localization (FDR = 3.97×10^{-10}), regulation of multicellular organismal process (FDR = 1.6×10^{-8}), cellular process (FDR = 1.31×10^{-7}), developmental process (FDR = 5.43×10^{-7}). When considering the 540 genes modulated by LUR treatment in CMS rats, the network analysis highlighted a statistically significant association among the DEGs ($p = 1 \times 10^{-16}$), with 415 nodes and 865 edges (expected number of edges: 539) (Supplementary Fig. S2). Enrichment analysis revealed the KEGG pathways potentially involved, including MAPK signalling (FDR = 0.0113) and calcium signalling (FRD = 0.0121) pathways. Furthermore, the network was significantly enriched for several GO biological processes, including regulation of biological quality (FDR = 7.85×10^{-9}), positive regulation of biological process (FDR = 6.51×10^{-8}), regulation of localization (FDR = 1.38×10^{-7}), regulation of synaptic plasticity (FDR = 1.38×10^{-7}) and regulation of multicellular organismal process (FDR = 9.39×10^{-7}). Full list of KEGG pathways and GO biological processes is reported in Supplementary Tables 5 and 6.

3.3. Lurasidone treatment reverses CMS-induced anhedonia by influencing cell viability

Next, we decided to compare the transcriptomic changes induced by CMS exposure, versus CT, with those found in CMS animals treated with lurasidone, versus CMS animals treated with the vehicle. By overlapping these two gene lists, we found 107 genes concurrently modulated in both comparisons (Fig. 4A). Heatmap depicting logFC of 107 DEGs is reported in Supplementary Fig. S3A and shows that the genes significantly upregulated by CMS are downregulated by lurasidone treatment and vice versa. The list of genes with relative logFC and pvalue is reported in Supplementary Table 7. In line, 16 pathways showed opposite prediction of activation (or inhibition) in our datasets (Supplementary Table 8). In detail, when CMS animals were compared to CT, 11 pathways were predicted to be inhibited and 5 pathways were predicted to be activated. Interestingly, all these pathways showed an opposite pattern of prediction when considering CMS rats treated with lurasidone compared to vehicle-treated animals. Among the above 16 enriched pathways, 6 were related to nervous system (in bold, Supplementary Table 8), namely Netrin, Oxytocin, Neurovascular Coupling, CREB, Endocannabinoid Neuronal Synapse and Dopamine-DARPP32 Feedback in cAMP Signalling pathways (Fig. 4B). Only the netrin signalling pathway displayed $|z| > 2$. In particular, we found that the netrin signalling was predicted to be inhibited by CMS exposure (z-score = −2) while lurasidone treatment was predicted to activate this pathway (z-score = 2). Actin binding LIM protein family member 2 (*Ablim2*), calcium voltage-gated channel subunit alpha1 E (*Cacna1e*), calcium voltage-gated channel subunit alpha1 G (*Cacna1g*) and unc-5 netrin receptor A (*Unc5a*) belong to this pathway and were all downregulated in the PFC of CMS animals, while lurasidone treatment could rescue these alterations and induced an increase in their expression levels. Similarly, all the other pathways, except for Neurovascular Coupling Signalling, were predicted to be inhibited in CMS animals versus CT and activated in CMS lurasidone-treated animals compared to CMS vehicle-treated animals.

Next, enriched biological functions were identified (Supplementary

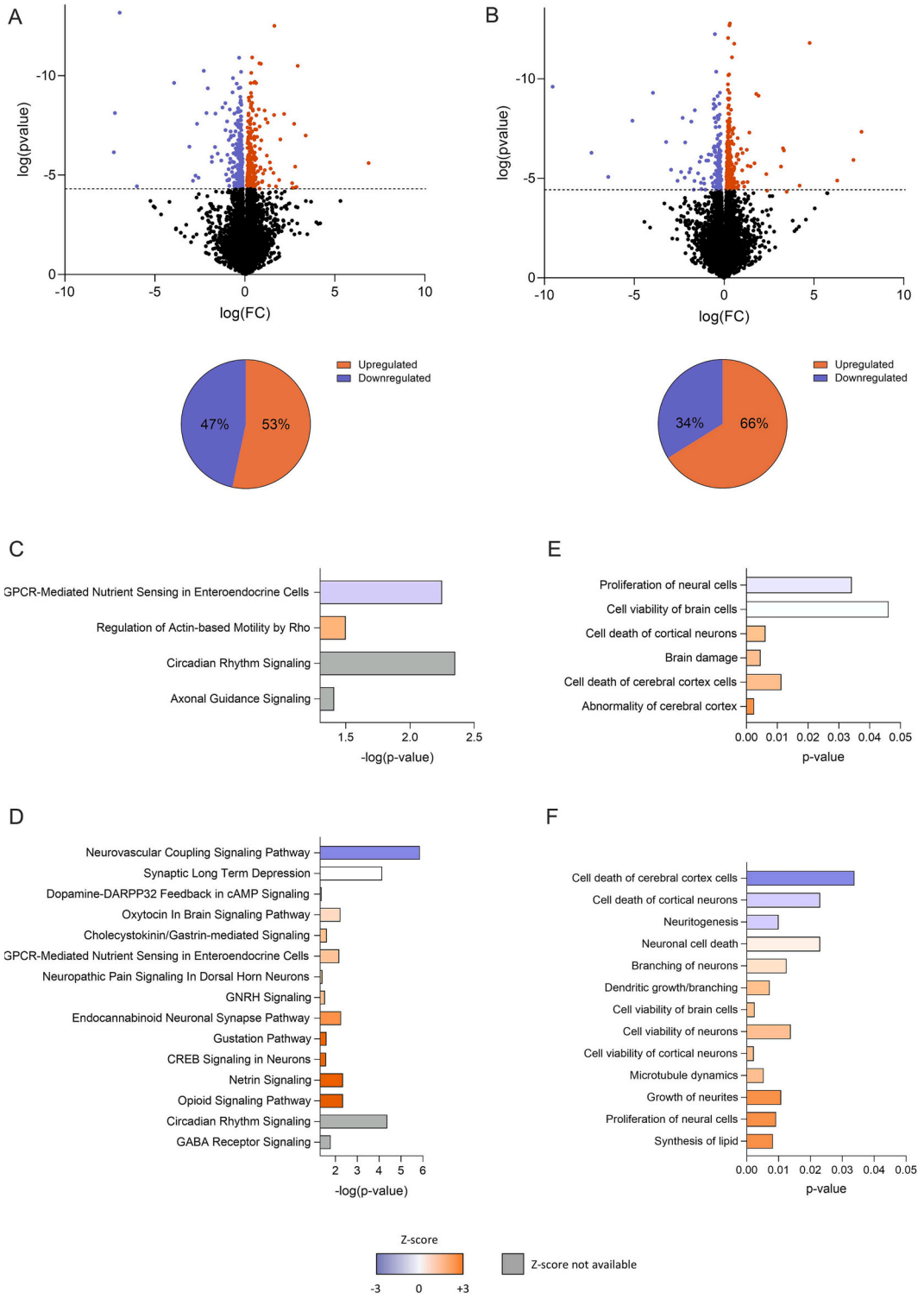


Fig. 3. Transcriptomic analysis of the PFC of adult male rats exposed to CMS and chronically treated with lurasidone.
Volcano plot representation of differential analysis of genes and the percentage of up- and down-regulated genes in the PFC of CMS animals treated with vehicle as compared to CT (panel A) and of CMS animals treated with lurasidone as compared to CMS animals treated with vehicle (panel B). The DEGs are displayed in blue (FC < -1.1) or orange (FC > 1.1). Bar plot representation of significant modulated pathways in the PFC of CMS animals treated with vehicle as compared to CT (panel C) and of CMS animals treated with lurasidone as compared to CMS animals treated with vehicle (panel D).
Bar plot representation of significant biological functions modulated in the PFC of CMS animals treated with vehicle as compared to CT (panel E) and of CMS animals treated with lurasidone as compared to CMS animals treated with vehicle (panel F).

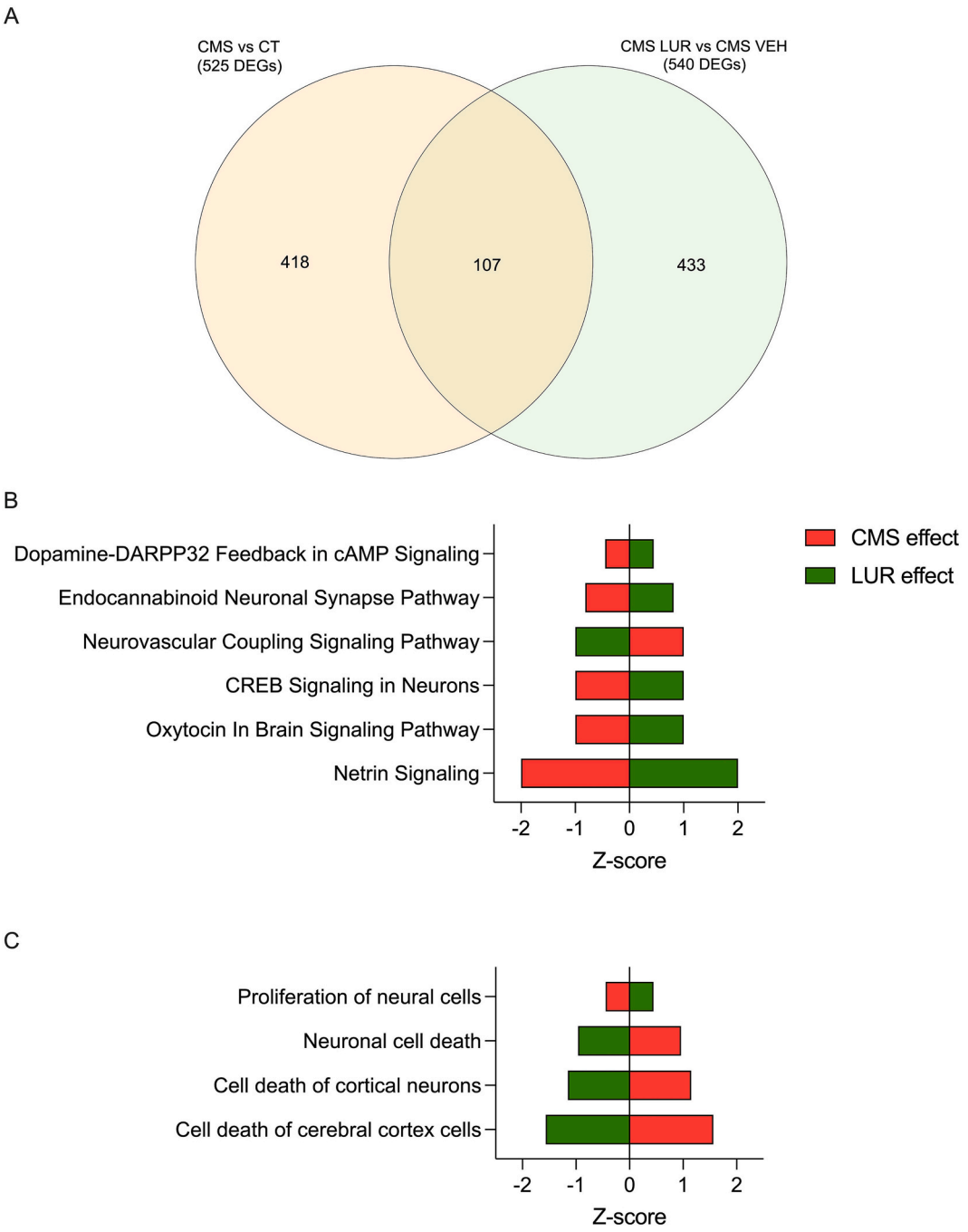


Fig. 4. Comparison analysis of DEGs in the PFC of CMS animals as compared to CT and of CMS animals treated with lurasidone as compared to CMS animals treated with vehicle.
Venn's diagram of genes modulated in the PFC by CMS exposure vs. CT and by lurasidone treatment in CMS rats vs CMS VEH (panel A). Bar plot representation of significant pathways (panel B) and biological functions (panel C) modulated by both CMS and lurasidone.

Table 9). The analysis confirmed the involvement of cell death and survival mechanisms (Fig. 4C). Indeed, we found that neuronal cell death was predicted to be stimulated in CMS animals, when compared to CT, while CMS rats treated with lurasidone show an opposite pattern, with a prediction of inhibition. At the same time, proliferation of neural cells was predicted to be inhibited by CMS exposure and upregulated by lurasidone treatment.

In addition, Venn's analysis revealed 433 genes differentially modulated by lurasidone treatment in CMS animals, that were not altered by CMS exposure (Fig. 4A), which may represent genes that are not altered by CMS exposure but could be regulated by drug treatment. We found 12 pathways (Supplementary Table 10), 5 of which are related to the nervous system. One pathway was upregulated (Opioid Signalling Pathway), 1 pathway had a z-score equal to zero, 1 pathway was downregulated (Neurovascular Coupling Signalling Pathway), and 2 pathways did not have available z-scores. Although not related to the nervous system, the White Adipose Tissue Browning Pathway was the only one reaching $|z| > 2$. We also identified 6 enriched biological functions (Supplementary Table 11) among which the extension of neurites and the synthesis of lipids showed the strongest modulation and were predicted to be upregulated by lurasidone treatment (z-score = 2 and z-score = 1.98, respectively).

3.4. Analysis of genes and pathways impaired by CMS exposure, which were not reverted by lurasidone

Next, in order to identify the genes and related pathways affected by CMS exposure, which were not modulated by chronic lurasidone treatment, we considered the 418 genes that were significantly modulated only by CMS exposure (Fig. 4A). Such gene are involved in 30 pathways (Supplementary Table 12), although only 2 of them were related to the nervous system. Specifically, the serotonin receptor signalling was predicted to be downregulated while the regulation of actin-based motility by Rho showed a prediction of activation. Although not related to the nervous system, the Granzyme A Signalling and Oxidative Phosphorylation pathways were the only ones showing a z-score < -2 and > 2, respectively. We also investigated possible biological functions involved, although the software was not able to calculate a prediction of activation or inhibition for any of them (Supplementary Table 13).

Last, we overlapped the genes differentially expressed in CMS animals treated with vehicle, as compared to CT, with the genes differentially expressed in CMS animals treated with lurasidone, as compared to CT. We identified 73 DEGs (Fig. 5A). The Heatmap depicting logFC is reported in Supplementary Fig. S3B showing that all the genes upregulated (or downregulated) in CMS vehicle-treated rats were similarly upregulated (or downregulated) in CMS lurasidone-treated animals, suggesting that the pharmacological treatment did not modulate the changes produced by CMS exposure. The list of genes with relative logFC and pvalue is reported in Supplementary Table 13. The pathway analysis revealed 19 enriched pathways (Supplementary Table 14). However, a prediction of activation or inhibition was available only for 1 pathway, namely G-Protein Coupled Receptor Signalling Pathway. In detail, the comparison analysis showed an activation of this pathway in both CMS animals treated with vehicle, as compared to CT animals, and in CMS lurasidone-treated animals, as compared to CT (Fig. 5B). Moreover, we found 4 biological functions involved, although the software was not able to calculate a prediction of activation or inhibition (Supplementary Table 15).

4. Discussion

In the present study, we performed RNA sequencing in the PFC to identify transcriptional profiles associated with the susceptibility to CMS exposure as well as with the ability of lurasidone in counteracting stress-induced alterations. In addition to confirming the efficacy of lurasidone in normalizing the anhedonic phenotype produced by CMS exposure, our results revealed key biological mechanisms altered in rats exposed to CMS. Furthermore, we reported that some of these alterations are modulated in an opposite direction by lurasidone administration.

Previous studies have clearly shown that different antidepressant drugs are able to revert the deficits induced by CMS exposure (Paladini et al., 2021; Rossetti et al., 2016; Willner, 2017). Similarly, we have previously shown that the antipsychotic drug lurasidone can normalize the behavioral defects produced by CMS exposure, including anhedonia that represents a key pathologic domain in different mental disorders (Begni et al., 2022; Calabrese et al., 2020; Luoni et al., 2015; Rossetti et al., 2018). The present results suggest that the netrin signalling pathway may represent one of the most relevant systems involved in the detrimental consequences of CMS exposure as well as in the therapeutic effects of lurasidone treatment within the PFC (Fig. 6 A and 6 B, respectively). More specifically, we found a prediction of inhibition of netrin signalling pathway as a consequence of CMS exposure that lurasidone was able to counteract. The netrin signalling pathway is involved in the fine organization of neuronal circuits achieved during perinatal life that comprises dynamic mechanisms of remodelling of the actin cytoskeleton essential for the proper neuronal morphology and synaptic plasticity. In addition, it has been found implicated in regulating synapse function and activity-dependent synaptic plasticity in the adult brain (Glasgow et al., 2021). The netrin family acts as guidance cue, regulating the growth of axons and dendrites toward their targets (Lanoue and Cooper, 2019). Downstream effectors of Netrin comprise its receptors Deleted in Colorectal Cancer (DCC) and UNC5 as well as the UNC-115/Ablim family of actin-biding proteins and the modulatory protein Enabled (Gitai et al., 2003).

Epidemiological studies have reported an association between polymorphisms in *DCC* and *netrin-1* genes and depression (Torres-Berrío et al., 2020). Likewise, genetic variants in *DCC* have been found associated with schizophrenia (Vosberg et al., 2020). Furthermore, both depressed subjects and adult animals exposed to a chronic stress model of depression exhibited higher *DCC* mRNA levels in the PFC (Torres-Berrío et al., 2020). Similarly, chronic stress during adolescence dysregulated *netrin-1/DCC* expression in mesolimbic DA regions (Vassilev et al., 2021). An altered methylation status of the *netrin-1* gene has been shown in association with depression in a genome-wide methylation study of twins (Roberson-Nay et al., 2020). In line, genetic polymorphisms in the *UNC5C* gene have been identified in both schizophrenic and depressed subjects (Tang et al., 2019). Our data specifically showed a downregulation of *UNC5A* and *Ablim2* genes expression following CMS exposure, while the treatment with lurasidone could

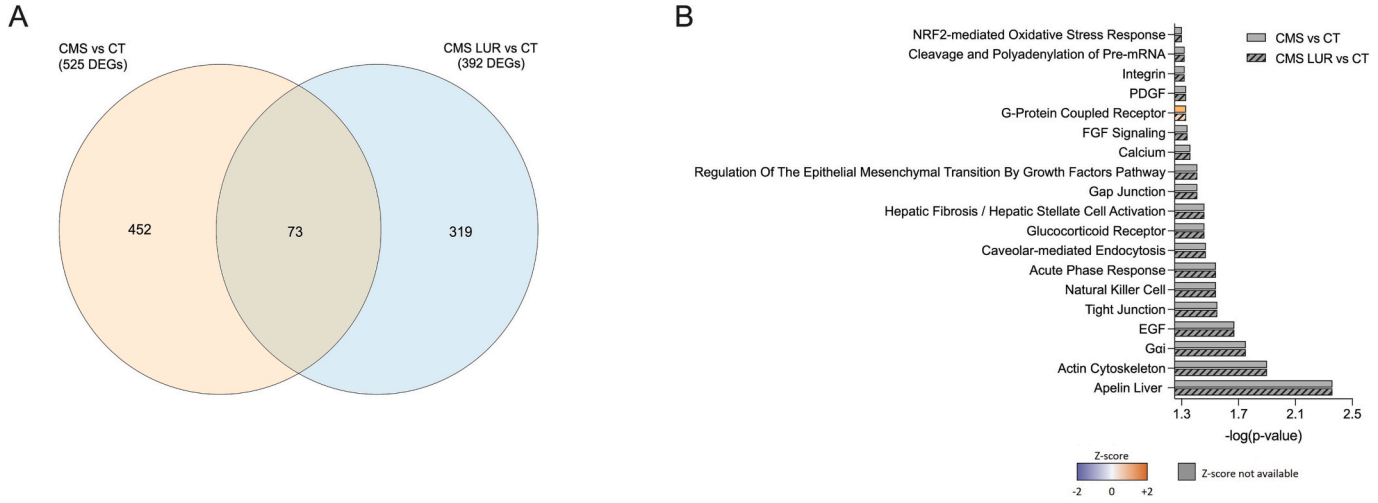


Fig. 5. Comparison analysis of DEGs in the PFC of CMS animals as compared to CT and of CMS animals treated with lurasidone as compared to CT.
Venn's diagram of DEGs modulated in the PFC by CMS exposure vs. CT and by lurasidone treatment in CMS rats vs CT (panel A). Bar plot representation of significant modulated pathways in the PFC of CMS animals treated either with vehicle or lurasidone, as compared to CT (panel B).

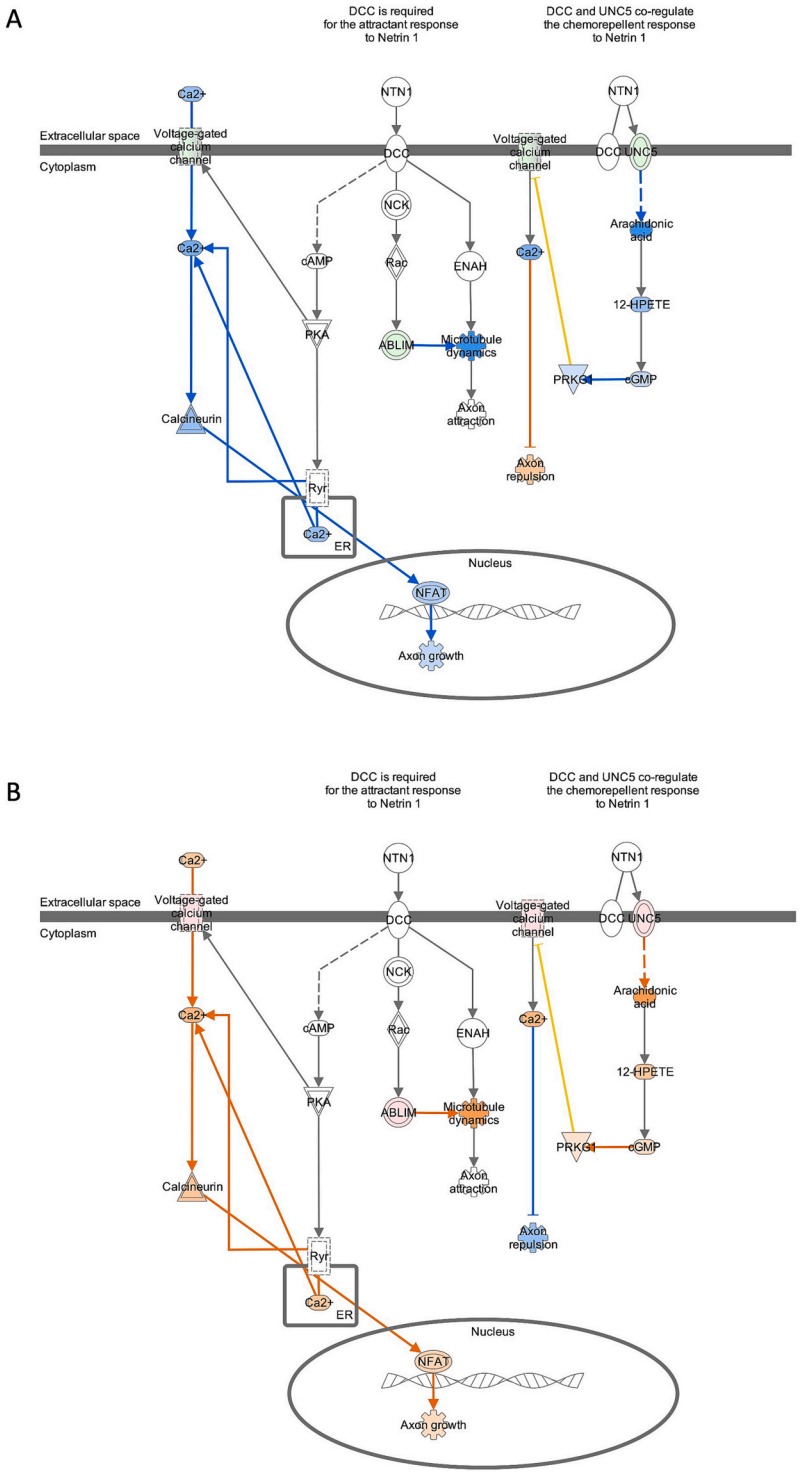


Fig. 6. Netrin signalling pathway.
Modulation of the netrin pathway in the PFC of CMS animals as compared to CT rats (panel A) and of CMS animals treated with lurasidone as compared to CMS animals treated with vehicle (panel B). Green: downregulated genes; Red: upregulated genes; Blue: genes predicted to be inhibited; Orange: genes predicted to be activated.

revert this modulation and increased their mRNA levels.

It has been shown that neural remodelling involves 5-HT7 receptors, contributing to the modulation of synaptic plasticity and neuronal connectivity during both the developing and mature brain (Crispino et al., 2020). Furthermore, it has been recently discovered that 5-HT7R induces activation of matrix metalloproteinase 9 (MMP-9), followed by CD44 cleavage and consequent cell division cycle protein 42 (Cdc42) activation. Elevated blood levels of MMP-9 have been found in patients with depression as well as in preclinical models of chronic stress (Rybakowski et al., 2013; van der Kooij et al., 2014). In line, MMP-9 inhibition prevents stress-induced behavioral alterations, while 5-HT7R agonist-mediated MMP-9 activation leads to depressive-like behaviours (van der Kooij et al., 2014). It is important to highlight that, compared to other antipsychotic drugs, lurasidone has high binding

activity to 5-HT7R (Nakazawa et al., 2013).

Together these data suggest that the ability of lurasidone to inhibit synaptic pruning, by antagonising 5HTR-7, associated with a stimulation of the netrin signalling pathway responsible for the correct axon guidance and elongation, may account for the therapeutical properties of lurasidone.

In agreement with this possibility, the analysis of the biological functions modulated by stress exposure and drug treatment suggests the involvement of cell death and survival mechanisms. Indeed, CMS exposure may induce cell death within the PFC, an effect that can be counteracted by lurasidone through a stimulation of neuronal proliferation. Among the genes involved in such processes, ABL Proto-Oncogene 1 (*Abl1*), nerve growth factor receptor (*Ngfr*) and Ribosomal Protein S6 (*Rps6*) showed the largest changes. Abl1 is involved in cell proliferation or differentiation, survival or death, retraction or migration. Within the brain, it has been shown to play an important role in neuronal development (Schlatterer et al., 2011) and it has been linked to neuronal degeneration and cell death (J. Y. J. Wang, 2014). Moreover, previous studies found that Abl1 is upregulated in the blood of gastric cancer and colorectal cancer patients with depression (Huang et al., 2019; Wei et al., 2009). In line, we found that CMS exposure led to an upregulation of *Abl1* that could be counteracted by chronic lurasidone administration.

The *Ngfr* gene encodes the p75 neurotrophin receptor (p75NTR) that is involved in neuronal death via the activation of proBDNF-mediated apoptotic signalling (Lee et al., 2001). In line, previous research showed that Ngfr expression is increased in the blood of patients with depression as well as in the brain of animals exposed to chronic stress (Bai et al., 2016; L. Zhou et al., 2013). We have previously shown that CMS exposure could downregulate the BDNF/proBDNF ratio and chronic treatment with lurasidone was capable to revert and normalize it (Luoni et al., 2015). Likewise, in this study, we found that lurasidone could revert the upregulation of *Ngfr* mRNA levels in the PFC of stressed animals. However, a possible modulation of Ngfr by lurasidone has never been evaluated and further investigations need to be conducted.

Rps6 phosphorylation is commonly used as a marker for neuronal activity and synaptic plasticity. In line with preclinical and clinical studies showing reduced size and activity of PFC in depression (X.-T. Zhou et al., 2020), we found that CMS exposure strongly reduced the expression of *RpS6*, while lurasidone administration upregulated its mRNA levels. Reduced Rps6 phosphorylation has previously been reported in the hippocampus of mice exposed to CMS (Chevalier et al., 2020). Similarly, a decrease of Rps6 expression and phosphorylation has been found in the brain of subjects with schizophrenia (Ibarra-Lecue et al., 2020). Interestingly, an acute administration of ketamine increased Rps6 phosphorylation (Tedesco et al., 2013), while no changes were observed in the brain of rats chronically treated with different antipsychotic drugs, including haloperidol, clozapine, or risperidone (Ibarra-Lecue et al., 2020).

In addition, the transcriptomic analysis revealed that lurasidone administration in CMS-exposed animals modulates other pathways, including the gustation signalling pathway. The genes involved in the gustation pathway comprised some receptors and channels such as the calcium voltage-gated channel, the GABA receptor, the potassium voltage-gated channel, the acid-sensing ion channel 1 (Asic1) and the pannexin 1 channel (Panx1). Panx1 is the most ubiquitous subtype of the pannexin family expressed in the brain where it allows the communication between intra- and extracellular compartments and mediates the release of signalling molecules such as the main energy substrate ATP into the extracellular space. In neurons, Panx1 is implicated in electrical communication, short-term memory formation, proliferation and migration of neural stem cells and apoptotic signalling (Chekeni et al., 2010; Wicki-Stordeur et al., 2012). Active Panx1 has been documented under pathological conditions such as brain ischemic injury, withdrawal syndrome and seizure, while an inhibition of Panx1 ameliorated the outcomes (Burma et al., 2017; Dvoriantchikova et al., 2012; Santiago et al., 2011). Some studies investigated the contribution of pannexins in mental disorders. Adult Panx1$^{-/-}$ mice showed enhanced anxiety and impaired hippocampus-dependent object recognition and spatial memory (Prochnow et al., 2012). Likewise, mice subjected to chronic social defeat stress displayed decreased expression and function of Panx1 channel in the PFC (Ni et al., 2018). Pharmacological blockade of Panx1 within the PFC induced depressive-like phenotypes and increased stress susceptibility (Ni et al., 2018). Similarly, a pharmacological inhibition of Panx1 within the nucleus accumbens enhanced the vulnerability to stress exposure, while it had no effect if infused into the ventral hippocampus (Heshmati et al., 2016). Our data confirmed the results observed in the PFC, indicating that lurasidone upregulated the expression of *Panx1* in CMS animals compared to animals treated with vehicle.

Asic1 is a cation-selective H + -gated channel and is extensively expressed in the central nervous system where it is thought to regulate synaptic plasticity (Storozhuk et al., 2021). Preclinical studies reported its involvement in fear extinction in the ventral hippocampus (Q. Wang et al., 2018) as well as in depression-related behaviours within the amygdala (Coryell et al., 2009). However, whether and how Asic1 plays a role in other brain regions such as the PFC has not been established yet. Moreover, it seems that the antidepressant properties of other drugs such as fluoxetine, desipramine and bupropion do not involve Asic1 activity (Coryell et al., 2009).

CREB signalling, an important mechanism connecting synaptic function and gene transcription, was also among the pathways modulated by CMS and lurasidone treatment. Indeed, we found that CREB pathway was downregulated by CMS exposure, an effect that was reverted by the antipsychotic drug treatment. These results are in line with a number of previous reports showing that CREB activity is reduced upon stress exposure or in depressive-like conditions (Qi et al., 2008; Wallace et al., 2009; Y. Xu et al., 2006). Moreover, the expression and activity of CREB appears to be up-regulated after chronic antidepressant administration (Blendy, 2006; Gass and Riva, 2007). It is interesting to note that Panx1 presents a binding site for CREB (Boyce et al., 2018), which may strengthen the relevance of these mechanisms in stress and therapeutic responses.

Lastly, the opioid signalling pathway resulted to be induced by lurasidone treatment. The opioid system has been largely implicated in emotional processes and depression (Jelen et al., 2022). Accordingly, opioid drugs have shown antidepressant properties in both preclinical and clinical studies. However, additional work is needed to comprehend the contribution of the opioid system in modulating depressive-like conditions (Jelen et al., 2022).

A more global view at these data would suggest that CMS exposure affects the transcriptional levels of genes involved in neuronal proliferation (*Abl1*, *Ngfr*, *Rps6*) and elongation (*UNC5A* and *Ablim2*), ultimately leading to deficits in synaptic plasticity, as confirmed by the modulation of CREB signalling pathway. On the contrary, lurasidone, probably with the contribution of its modulation of 5HTR-7 receptors, appears to counteract these transcriptional changes, eventually promoting neural survival and plasticity, and contributing to the recovery from the anhedonic condition. Furthermore, these data suggest that lurasidone may simultaneously act on other pathways that were not modulated by CMS, as the gustation and opioid signalling pathways, which have also been linked to neural proliferation (Persson et al., 2003; Wicki-Stordeur et al., 2012).

We must acknowledge some limitations of our study. Since we wanted to expand our knowledge on the molecular changes brought by lurasidone in CMS rats, we only focused on the PFC. It will be important to investigate the adaptive changes in other brain regions, such as NAc and AMY, which are functionally associated with the PFC. Another limitation of our study is that we focused only on male animals and, considering the sexual dimorphism of mental disorders, it will be important to establish similarities and differences in males and females exposed to CMS, as well as potential differences in the responsiveness to drug treatment.

5. Conclusions

Altogether, although extensive clinical and preclinical research has been conducted so far on the therapeutic effects of lurasidone, our study provides novel data on the downstream pathways and functions modulated by a chronic treatment with lurasidone within the PFC that plays a key role in the development and manifestation of mental disorders (Duman et al., 2016; Liu et al., 2017; McEwen and Morrison, 2013). We suggest that the multi-receptor binding profile of lurasidone could converge onto specific signalling pathways within this brain area and provides therefore a starting point to thoroughly evaluate these mechanisms, eventually aiming to develop more effective treatments for depression.

Ethical statement

All procedures included in this study are in conformity with the rules and principles of the 86/609/EEC Directive and have been approved by the Local Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

Role of the funding source

Funding for this study was provided by the Italian Ministry of University and Research to M.A.R. (PON "Ricerca e Innovazione" PerMedNet project ARS01_01226) and from Sumitomo Pharma Co. to M.A.R. All funding bodies had no role in designing the study, in analysing and interpretating of data as well as in the writing of the manuscript and in the decision to submit it for publication.

CRediT authorship contribution statement

Veronica Begni: Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Moira Marizzoni:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Kerstin Camile Creutzberg:** Formal analysis, Investigation, Methodology. **Diana Morena Silipo:** Formal analysis, Investigation, Methodology, Writing – original draft. **Mariusz Papp:** Conceptualization, Project administration, Supervision. **Annamaria Cattaneo:** Conceptualization, Writing - review & editing. **Marco Andrea Riva:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

M.A.R. has received compensation as speaker/consultant from Angelini, Exeltis, Iqvia, Lundbeck, Otzuka, and Sumitomo Pharma, and he has received research grants from Sumitomo Pharma. All other authors declare that they have no conflicts of interest.

Data availability

Illumina sequencing reads are available upon request.

Acknowledgments

We are grateful to Sumitomo Pharma Co. Ltd. for the generous gift of lurasidone.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2023.110885.

References

Ali, Z., Tegin, C., El-Mallakh, R.S., 2020. Evaluating lurasidone as a treatment option for bipolar disorder. Expert. Opin. Pharmacother. 21 (3), 253–260. https://doi.org/10.1080/14656566.2019.1695777.

Arnsten, A.F.T., 2009. Stress signalling pathways that impair prefrontal cortex structure and function. Nat. Rev. Neurosci. 10 (6), 410–422. https://doi.org/10.1038/nrn2648.

Bagot, R.C., Cates, H.M., Purushothaman, I., Vialou, V., Heller, E.A., Yieh, L., LaBonté, B., Peña, C.J., Shen, L., Wittenberg, G.M., Nestler, E.J., 2017. Ketamine and imipramine reverse transcriptional signatures of susceptibility and induce resilience-specific gene expression profiles. Biol. Psychiatry 81 (4), 285–295. https://doi.org/10.1016/j.biopsych.2016.06.012.

Bai, Y.-Y., Ruan, C.-S., Yang, C.-R., Li, J.-Y., Kang, Z.-L., Zhou, L., Liu, D., Zeng, Y.-Q., Wang, T.-H., Tian, C.-F., Liao, H., Bobrovskaya, L., Zhou, X.-F., 2016. ProBDNF signaling regulates depression-like behaviors in rodents under chronic stress. Neuropsychopharmacology 41 (12), 2882–2892. https://doi.org/10.1038/npp.2016.100.

de Bartolomeis, A., Barone, A., Begni, V., Riva, M.A., 2022. Present and future antipsychotic drugs: a systematic review of the putative mechanisms of action for efficacy and a critical appraisal under a translational perspective. Pharmacol. Res. 176, 106078 https://doi.org/10.1016/j.phrs.2022.106078.

Begni, V., Pisano, I., Marizzoni, M., Marchisella, F., Creutzberg, K.C., de Rosa, F., Cattaneo, A., Gruca, P., Litwa, E., Papp, M., Riva, M.A., 2022. Exposure to chronic stress impairs the ability to cope with an acute challenge: modulation by lurasidone treatment. Eur. Neuropsychopharmacol. 61, 78–90. https://doi.org/10.1016/j.euroneuro.2022.06.005.

Blendy, J.A., 2006. The role of CREB in depression and antidepressant treatment. Biol. Psychiatry 59 (12), 1144–1150. https://doi.org/10.1016/j.biopsych.2005.11.003.

Boyce, A.K.J., Epp, A.L., Nagarajan, A., Swayne, L.A., 2018. Transcriptional and post-translational regulation of pannexins. Biochim. Biophys. Acta Biomembr. 1860 (1), 72–82. https://doi.org/10.1016/j.bbamem.2017.03.004.

Burma, N.E., Bonin, R.P., Leduc-Pessah, H., Baimel, C., Cairncross, Z.F., Mousseau, M., Shankara, J.V., Stemkowski, P.L., Baimoukhametova, D., Bains, J.S., Antle, M.C., Zamponi, G.W., Cahill, C.M., Borgland, S.L., de Koninck, Y., Trang, T., 2017. Blocking microglial pannexin-1 channels alleviates morphine withdrawal in rodents. Nat. Med. 23 (3), 355–360. https://doi.org/10.1038/nm.4281.

Calabrese, F., Brivio, P., Sbrini, G., Gruca, P., Lason, M., Litwa, E., Niemczyk, M., Papp, M., Riva, M.A., 2020. Effect of lurasidone treatment on chronic mild stress-induced behavioural deficits in male rats: the potential role for glucocorticoid receptor signalling. J. Psychopharmacol. 34 (4), 420–428. https://doi.org/10.1177/0269881119895547.

Chekeni, F.B., Elliott, M.R., Sandilos, J.K., Walk, S.F., Kinchen, J.M., Lazarowski, E.R., Armstrong, A.J., Penuela, S., Laird, D.W., Salvesen, G.S., Isakson, B.E., Bayliss, D.A., Ravichandran, K.S., 2010. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature 467 (7317), 863–867. https://doi.org/10.1038/nature09413.

Chevalier, G., Siopi, E., Guenin-Macé, L., Pascal, M., Laval, T., Rifflet, A., Boneca, I.G., Demangel, C., Colsch, B., Pruvost, A., Chu-Van, E., Messager, A., Leulier, F., Lepousez, G., Eberl, G., Lledo, P.-M., 2020. Effect of gut microbiota on depressive-like behaviors in mice is mediated by the endocannabinoid system. Nat. Commun. 11 (1), 6363. https://doi.org/10.1038/s41467-020-19931-2.

Coryell, M.W., Wunsch, A.M., Haenfler, J.M., Allen, J.E., Schnizler, M., Ziemann, A.E., Cook, M.N., Dunning, J.P., Price, M.P., Rainier, J.D., Liu, Z., Light, A.R., Langbehn, D.R., Wemmie, J.A., 2009. Acid-sensing Ion Channel-1a in the amygdala, a novel therapeutic target in depression-related behavior. J. Neurosci. 29 (17), 5381–5388. https://doi.org/10.1523/JNEUROSCI.0360-09.2009.

Creutzberg, K.C., Begni, V., Marchisella, F., Papp, M., Riva, M.A., 2023. Early effects of lurasidone treatment in a chronic mild stress model in male rats. Psychopharmacology 240 (4), 1001–1010. https://doi.org/10.1007/s00213-023-06343-5.

Crispino, M., Volpicelli, F., Perrone-Capano, C., 2020. Role of the serotonin receptor 7 in brain plasticity: from development to disease. Int. J. Mol. Sci. 21 (2), 505. https://doi.org/10.3390/ijms21020505.

DelBello, M.P., Goldman, R., Phillips, D., Deng, L., Cucchiaro, J., Loebel, A., 2017. Efficacy and safety of Lurasidone in children and adolescents with bipolar I depression: a double-blind, placebo-controlled study. J. Am. Acad. Child Adolesc. Psychiatry 56 (12), 1015–1025. https://doi.org/10.1016/j.jaac.2017.10.006.

Duman, R.S., Aghajanian, G.K., Sanacora, G., Krystal, J.H., 2016. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat. Med. 22 (3), 238–249. https://doi.org/10.1038/nm.4050.

Dvoriantchikova, G., Ivanov, D., Barakat, D., Grinberg, A., Wen, R., Slepak, V.Z., Shestopalov, V.I., 2012. Genetic ablation of Pannexin1 protects retinal neurons from ischemic injury. PLoS One 7 (2), e31991. https://doi.org/10.1371/journal.pone.0031991.

Gass, P., Riva, M.A., 2007. CREB, neurogenesis and depression. BioEssays 29 (10), 957–961. https://doi.org/10.1002/bies.20658.

Gitai, Z., Yu, T.W., Lundquist, E.A., Tessier-Lavigne, M., Bargmann, C.I., 2003. The netrin receptor UNC-40/DCC stimulates axon attraction and outgrowth through enabled and, in parallel, Rac and UNC-115/AbLIM. Neuron 37 (1), 53–65. https://doi.org/10.1016/S0896-6273(02)01149-2.

Glasgow, S.D., Ruthazer, E.S., Kennedy, T.E., 2021. Guiding synaptic plasticity: novel roles for netrin-1 in synaptic plasticity and memory formation in the adult brain. J. Physiol. 599 (2), 493–505. https://doi.org/10.1113/JP278704.

Herzog, D.P., Pascual Cuadrado, D., Treccani, G., Jene, T., Opitz, V., Hasch, A., Lutz, B., Lieb, K., Sillaber, I., van der Kooij, M.A., Tiwari, V.K., Müller, M.B., 2021. A distinct

transcriptional signature of antidepressant response in hippocampal dentate gyrus granule cells. Transl. Psychiatry 11 (1), 4. https://doi.org/10.1038/s41398-020-01136-2.
Heshmati, M., Golden, S.A., Pfau, M.L., Christoffel, D.J., Seeley, E.L., Cahill, M.E., Khibnik, L.A., Russo, S.J., 2016. Mefloquine in the nucleus accumbens promotes social avoidance and anxiety-like behavior in mice. Neuropharmacology 101, 351–357. https://doi.org/10.1016/j.neuropharm.2015.10.013.
Huang, T., Zhou, F., Yuan, X., Yang, T., Liang, X., Wang, Y., Tu, H., Chang, J., Nan, K., Wei, Y., 2019. Reactive oxygen species are involved in the development of gastric cancer and gastric cancer-related depression through ABL1-mediated inflammation signaling pathway. Oxidative Med. Cell. Longev. 2019, 1–12. https://doi.org/10.1155/2019/5813985.
Hulsen, T., de Vlieg, J., Alkema, W., 2008. BioVenn – a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. BMC Genomics 9 (1), 488. https://doi.org/10.1186/1471-2164-9-488.
Ibarra-Lecue, I., Diez-Alarcia, R., Morentin, B., Meana, J.J., Callado, L.F., Urigüen, L., 2020. Ribosomal protein S6 hypofunction in postmortem human brain links mTORC1-dependent signaling and schizophrenia. Front. Pharmacol. 11 https://doi.org/10.3389/fphar.2020.00344.
Jelen, L.A., Stone, J.M., Young, A.H., Mehta, M.A., 2022. The opioid system in depression. Neurosci. Biobehav. Rev. 140, 104800 https://doi.org/10.1016/j.neubiorev.2022.104800.
van der Kooij, M.A., Fantin, M., Rejmak, E., Grosse, J., Zanoletti, O., Fournier, C., Ganguly, K., Kalita, K., Kaczmarek, L., Sandi, C., 2014. Role for MMP-9 in stress-induced downregulation of nectin-3 in hippocampal CA1 and associated behavioural alterations. Nat. Commun. 5 (1), 4995. https://doi.org/10.1038/ncomms5995.
Lanoue, V., Cooper, H.M., 2019. Branching mechanisms shaping dendrite architecture. Dev. Biol. 451 (1), 16–24. https://doi.org/10.1016/j.ydbio.2018.12.005.
Lee, R., Kermani, P., Teng, K.K., Hempstead, B.L., 2001. Regulation of cell survival by secreted proneurotrophins. Science 294 (5548), 1945–1948. https://doi.org/10.1126/science.1065057.
Liu, W., Ge, T., Leng, Y., Pan, Z., Fan, J., Yang, W., Cui, R., 2017. The role of neural plasticity in depression: from Hippocampus to prefrontal cortex. Neural Plast. 2017, 1–11. https://doi.org/10.1155/2017/6871089.
Lopez, J.P., Lücken, M.D., Brivio, E., Karamihalev, S., Kos, A., de Donno, C., Benjamin, A., Yang, H., Dick, A.L.W., Stoffel, R., Flachskamm, C., Ressle, A., Roeh, S., Huettl, R.-E., Parl, A., Eggert, C., Novak, B., Yan, Y., Yeoh, K., Chen, A., 2022. Ketamine exerts its sustained antidepressant effects via cell-type-specific regulation of Kcnq2. Neuron 110 (14), 2283–2298.e9. https://doi.org/10.1016/j.neuron.2022.05.001.
Luoni, A., Macchi, F., Papp, M., Molteni, R., Riva, M.A., 2015. 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 (4) https://doi.org/10.1093/ijnp/pyu061.
Malhi, G.S., Mann, J.J., 2018. Depression. Lancet 392 (10161), 2299–2312. https://doi.org/10.1016/S0140-6736(18)31948-2.
McEwen, B.S., Morrison, J.H., 2013. The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course. Neuron 79 (1), 16–29. https://doi.org/10.1016/j.neuron.2013.06.028.
Nakazawa, S., Yokoyama, C., Nishimura, N., Horisawa, T., Kawasaki, A., Mizuma, H., Doi, H., Onoe, H., 2013. Evaluation of dopamine D2/D3 and serotonin 5-HT2A receptor occupancy for a novel antipsychotic, lurasidone, in conscious common marmosets using small-animal positron emission tomography. Psychopharmacology 225 (2), 329–339. https://doi.org/10.1007/s00213-012-2815-9.
Ni, M., He, J.-G., Zhou, H.-Y., Lu, X.-J., Hu, Y.-L., Mao, L., Wang, F., Chen, J.-G., Hu, Z.-L., 2018. Pannexin-1 channel dysfunction in the medial prefrontal cortex mediates depressive-like behaviors induced by chronic social defeat stress and administration of mefloquine in mice. Neuropharmacology 137, 256–267. https://doi.org/10.1016/j.neuropharm.2017.12.004.
Nøhr, A.K., Lindow, M., Forsingdal, A., Demharter, S., Nielsen, T., Buller, R., Moltke, I., Vitezic, M., Albrechtsen, A., 2021. A large-scale genome-wide gene expression analysis in peripheral blood identifies very few differentially expressed genes related to antidepressant treatment and response in patients with major depressive disorder. Neuropsychopharmacology 46 (7), 1324–1332. https://doi.org/10.1038/s41386-021-01002-9.
Paladini, M.S., Spero, V., Begni, V., Marchisella, F., Guidi, A., Gruca, P., Lason, M., Litwa, E., Papp, M., Riva, M.A., Molteni, R., 2021. Behavioral and molecular effects of the antipsychotic drug blonanserin in the chronic mild stress model. Pharmacol. Res. 163, 105330 https://doi.org/10.1016/j.phrs.2020.105330.
Persson, A.I., Thorlin, T., Bull, C., Zarnegar, P., Ekman, R., Terenius, L., Eriksson, P.S., 2003. Mu- and delta-opioid receptor antagonists decrease proliferation and increase neurogenesis in cultures of rat adult hippocampal progenitors. Eur. J. Neurosci. 17 (6), 1159–1172. https://doi.org/10.1046/j.1460-9568.2003.02538.x.
Prochnow, N., Abdulazim, A., Kurtenbach, S., Wildförster, V., Dvoriantchikova, G., Hanske, J., Petrasch-Parwez, E., Shestopalov, V.I., Dermietzel, R., Manahan-Vaughan, D., Zoidl, G., 2012. Pannexin1 stabilizes synaptic plasticity and is needed for learning. PLoS One 7 (12), e51767. https://doi.org/10.1371/journal.pone.0051767.
Qi, X., Lin, W., Li, J., Li, H., Wang, W., Wang, D., Sun, M., 2008. Fluoxetine increases the activity of the ERK-CREB signal system and alleviates the depressive-like behavior in rats exposed to chronic forced swim stress. Neurobiol. Dis. 31 (2), 278–285. https://doi.org/10.1016/j.nbd.2008.05.003.
Roberson-Nay, R., Lapato, D.M., Wolen, A.R., Lancaster, E.E., Webb, B.T., Verhulst, B., Hettema, J.M., York, T.P., 2020. An epigenome-wide association study of early-onset major depression in monozygotic twins. Transl. Psychiatry 10 (1), 301. https://doi.org/10.1038/s41398-020-00984-2.
Rossetti, A.C., Papp, M., Gruca, P., Paladini, M.S., Racagni, G., Riva, M.A., 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. https://doi.org/10.1016/j.phrs.2015.10.022.
Rossetti, A.C., Paladini, M.S., Colombo, M., Gruca, P., Lason-Tyburkiewicz, M., Tota-Glowczyk, K., Papp, M., Riva, M.A., Molteni, R., 2018. Chronic stress exposure reduces Parvalbumin expression in the rat Hippocampus through an imbalance of redox mechanisms: restorative effect of the antipsychotic lurasidone. Int. J. Neuropsychopharmacol. 21 (9), 883–893. https://doi.org/10.1093/ijnp/pyy046.
Rybakowski, J.K., Remlinger-Molenda, A., Czech-Kucharska, A., Wojcicka, M., Michalak, M., Losy, J., 2013. Increased serum matrix metalloproteinase-9 (MMP-9) levels in young patients during bipolar depression. J. Affect. Disord. 146 (2), 286–289. https://doi.org/10.1016/j.jad.2012.07.019.
Sanson, A., Riva, M.A., 2020. Anti-stress properties of atypical antipsychotics. Pharmaceuticals 13 (10), 322. https://doi.org/10.3390/ph13100322.
Santiago, M.F., Veliskova, J., Patel, N.K., Lutz, S.E., Caille, D., Charollais, A., Meda, P., Scemes, E., 2011. Targeting Pannexin1 improves seizure outcome. PLoS One 6 (9), e25178. https://doi.org/10.1371/journal.pone.0025178.
Schlatterer, S.D., Acker, C.M., Davies, P., 2011. C-Abl in neurodegenerative disease. J. Mol. Neurosci. 45 (3), 445–452. https://doi.org/10.1007/s12031-011-9588-1.
Schmitt, A., Malchow, B., Hasan, A., Falkai, P., 2014. The impact of environmental factors in severe psychiatric disorders. Front. Neurosci. 8 https://doi.org/10.3389/fnins.2014.00019.
Storozhuk, M., Cherninskyi, A., Maximyuk, O., Isaev, D., Krishtal, O., 2021. Acid-sensing ion channels: focus on physiological and some pathological roles in the brain. Curr. Neuropharmacol. 19 (9), 1570–1589. https://doi.org/10.2174/1570159X19666210125151824.
Stupnikov, A., McInerney, C.E., Savage, K.I., McIntosh, S.A., Emmert-Streib, F., Kennedy, R., Salto-Tellez, M., Prise, K.M., McArt, D.G., 2021. Robustness of differential gene expression analysis of RNA-seq. Comput. Struct. Biotechnol. J. 19, 3470–3481. https://doi.org/10.1016/j.csbj.2021.05.040.
Tang, J., Chen, X., Cai, B., Chen, G., 2019. A logical relationship for schizophrenia, bipolar, and major depressive disorder. Part 4: evidence from chromosome 4 high-density association screen. J. Comp. Neurol. 527 (2), 392–405. https://doi.org/10.1002/cne.24543.
Tarazi, F.I., Riva, M.A., 2013. The preclinical profile of lurasidone: clinical relevance for the treatment of schizophrenia. Expert Opin. Drug Discovery 8 (10), 1297–1307. https://doi.org/10.1517/17460441.2013.815163.
Tedesco, V., Ravagnani, C., Bertoglio, D., Chiamulera, C., 2013. Acute ketamine-induced neuroplasticity. NeuroReport 24 (7), 388–393. https://doi.org/10.1097/WNR.0b013e32836131ad.
Torres-Berrío, A., Hernandez, G., Nestler, E.J., Flores, C., 2020. The Netrin-1/DCC guidance cue pathway as a molecular target in depression: translational evidence. Biol. Psychiatry 88 (8), 611–624. https://doi.org/10.1016/j.biopsych.2020.04.025.
Vassilev, P., Pantoja-Urban, A.H., Giroux, M., Nouel, D., Hernandez, G., Orsini, T., Flores, C., 2021. Unique effects of social defeat stress in adolescent male mice on the netrin-1/DCC pathway, prefrontal cortex dopamine and cognition. Eneuro 8 (2). https://doi.org/10.1523/ENEURO.0045-21.2021. ENEURO.0045-21.2021.
Vosberg, D.E., Leyton, M., Flores, C., 2020. The Netrin-1/DCC guidance system: dopamine pathway maturation and psychiatric disorders emerging in adolescence. Mol. Psychiatry 25 (2), 297–307. https://doi.org/10.1038/s41380-019-0561-7.
Wallace, D.L., Han, M.-H., Graham, D.L., Green, T.A., Vialou, V., Iñiguez, S.D., Cao, J.-L., Kirk, A., Chakravarty, S., Kumar, A., Krishnan, V., Neve, R.L., Cooper, D.C., Bolaños, C.A., Barrot, M., McClung, C.A., Nestler, E.J., 2009. CREB regulation of nucleus accumbens excitability mediates social isolation–induced behavioral deficits. Nat. Neurosci. 12 (2), 200–209. https://doi.org/10.1038/nn.2257.
Wang, J.Y.J., 2014. The capable ABL: what is its biological function? Mol. Cell. Biol. 34 (7), 1188–1197. https://doi.org/10.1128/MCB.01454-13.
Wang, Q., Wang, Q., Song, X.-L., Jiang, Q., Wu, Y.-J., Li, Y., Yuan, T.-F., Zhang, S., Xu, N.-J., Zhu, M.X., Li, W.-G., Xu, T.-L., 2018. Fear extinction requires ASIC1a-dependent regulation of hippocampal-prefrontal correlates. Sci. Adv. 4 (10) https://doi.org/10.1126/sciadv.aau3075.
Wei, Y., Zhou, F., He, D., Bai, J., Hui, L., Wang, X., Nan, K., 2009. The level of oxidative stress and the expression of genes involved in DNA-damage signaling pathways in depressive patients with colorectal carcinoma. J. Psychosom. Res. 66 (3), 259–266. https://doi.org/10.1016/j.jpsychores.2008.09.001.
Wicki-Stordeur, L.E., Dzugalo, A.D., Swansburg, R.M., Suits, J.M., Swayne, L.A., 2012. Pannexin 1 regulates postnatal neural stem and progenitor cell proliferation. Neural Dev. 7 (1), 11. https://doi.org/10.1186/1749-8104-7-11.
Willner, P., 2017. The chronic mild stress (CMS) model of depression: history, evaluation and usage. Neurobiol. Stress 6, 78–93. https://doi.org/10.1016/j.ynstr.2016.08.002.
World Health Organization, 2022. World Mental Health Report: Transforming Mental Health for all.
Xu, Y., Ku, B., Tie, L., Yao, H., Jiang, W., Ma, X., Li, X., 2006. Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. Brain Res. 1122 (1), 56–64. https://doi.org/10.1016/j.brainres.2006.09.009.
Xu, P., Chen, A., Li, Y., Xing, X., Lu, H., 2019. Medial prefrontal cortex in neurological diseases. Physiol. Genomics 51 (9), 432–442. https://doi.org/10.1152/physiolgenomics.00006.2019.

Zhou, L., Xiong, J., Lim, Y., Ruan, Y., Huang, C., Zhu, Y., Zhong, J., Xiao, Z., Zhou, X.-F., 2013. Upregulation of blood proBDNF and its receptors in major depression. J. Affect. Disord. 150 (3), 776–784. https://doi.org/10.1016/j.jad.2013.03.002.

Zhou, X.-T., Bao, W.-D., Liu, D., Zhu, L.-Q., 2020. Targeting the neuronal activity of prefrontal cortex: new directions for the therapy of depression. Curr. Neuropharmacol. 18 (4), 332–346. https://doi.org/10.2174/1570159X17666191101124017.

Zygmunt, M., Piechota, M., Rodriguez Parkitna, J., Korostyński, M., 2019. Decoding the transcriptional programs activated by psychotropic drugs in the brain. Genes Brain Behav. 18 (4), e12511 https://doi.org/10.1111/gbb.12511.