Chronic stress and impaired glutamate function elicit a depressive-like phenotype and common changes in gene expression in the mouse frontal cortex

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Abstract

Major depression might originate from both environmental and genetic risk factors. The environmental chronic mild stress (CMS) model mimics some environmental factors contributing to human depression and induces anhedonia and helplessness. Mice heterozygous for the synaptic vesicle protein (SVP) vesicular glutamate transporter 1 (VGLUT1) have been proposed as a genetic model of deficient glutamate function linked to depressive-like behaviour. Here, we aimed to identify, in these two experimental models, gene expression changes in the frontal cortex, common to stress and impaired glutamate function.

Both VGLUT1+/- and CMS mice showed helpless and anhedonic-like behavior. Microarray studies in VGLUT1+/- mice revealed regulation of genes involved in apoptosis, neurogenesis, synaptic transmission, protein metabolic process or learning and memory. In addition, RT-PCR studies confirmed gene expression changes in several glutamate, GABA, dopamine and serotonin neurotransmitter receptors. On the other hand, CMS affected the regulation of 147 transcripts, some of them involved in response to stress and oxidoreductase activity. Interestingly, 52 genes were similarly regulated in both models. Specifically, a dowregulation in genes that promote cell proliferation (**Anapc7**), cell growth (**CsnK1g1**), cell survival (**Hdac3**), inhibition of apoptosis (**Dido1**) was observed. Genes linked to cytoskeleton (**Hspg2**, **Invs**), psychiatric disorders (**Grin1**, **MapK12**) or an antioxidant enzyme (**Gpx2**) were also downregulated. Moreover, genes that inhibit the MAPK pathways (**Dusp14**), stimulate oxidative metabolism (**Eif4a2**) and enhance glutamate transmission (**Rab8b**) were upregulated.

We suggest that these genes could form part of the altered “molecular context” underlying depressive-like behaviour in animal models. The clinical relevance of these findings is discussed.

1. Introduction

Increasing evidence suggests that major depression might originate from both environmental and genetic risk factors. This observation early prompted the need to better refine and develop mouse-specific models for analysing depression. For instance, the chronic mild stress (CMS) model of depression was developed (Willner, 2005) in an attempt to mimic some of the environmental factors contributing to the induction of depressive disorders in humans (Kessler, 1997; Kendler et al., 1999; 2001; Monroe et al., 2006).

CMS reproduces core clinical symptoms such as long-lasting anhedonia and helplessness (Elizalde et al., 2008). In addition, CMS induces neuroadaptive changes that could be addressing clinical findings with depressed patients (Gould et al., 2007; Sanacora et al., 2004; Rajkowska., 2000; Lucassen et al., 2006; Frodl et al., 2008).

Recent clinical (Uezato et al., 2009) and preclinical studies (Tordera et al., 2007; García-García et al., 2009) have linked decreased levels of the synaptic vesicle protein (SVP) vesicular glutamate transporter 1 (VGLUT1) to depressive like behaviour. Specifically, decreased VGLUT1 levels in the frontal cortex of depressed subjects have been reported (Uezato et al., 2009) in post-mortem studies. In addition, recent studies with the VGLUT1 heterozygous mice (VGLUT1+/-) suggest that decreased VGLUT1 levels affects glutamate transmission (Balschun et al., 2009) and induces depressive-like behavior comorbid with anxiety and impaired recognition memory (Tordera et al., 2007). Moreover, VGLUT1+/- mice show decreased cortical

and hippocampal levels in GABA as well as an increased vulnerability to depressive-like behaviour after chronic stress (García-García et al., 2009).

Here, we aimed to study, using microarray technology, how decreased VGLUT1 levels (VGLUT1+/- mice) or an environmental model (chronic mild stress) affect behaviour and gene expression in the frontal cortex.

Firstly, anhedonic and helpless behaviour in both VGLUT1+/- and CMS models was comparatively studied. Secondly, we evaluated differences in gene expression in VGLUT1+/- mice compared to WT. From all the significant genes, we selected some candidates to validate by RT-PCR considering their relationship with different neurotransmitter systems and the coincidence with another genetic animal model of depression, the CB1 KO mice (Aso et al., 2010, Special Issue). Differences in gene expression in the CMS model were also evaluated. Subsequently, gene expression changes common to the VGLUT1+/- and CMS models were selected and their possible involvement in depressive-like behaviour was discussed.

2. Experimental procedures

2.1 Animals

Heterozygous VGLUT1 male mice (VGLUT1+/-) C57BL/6N were bred from heterozygous fathers (Dr S. Wojcik, Gottingen, Germany) and WT mothers (Harlan, France). The VGLUT1-/- knock-out allele was generated by truncation of the coding region of the VGLUT1 gene between the start codon and a *Bgl*II site in the fifth coding exon through homologous recombination in embryonic stem cells (129/ola background) (Wojcik et al., 2004). These mice show a progressive neuropathological phenotype and increased lethality rate at 2–3 weeks after birth. Mice were weaned and genotyped at the age of 3 weeks. VGLUT1+/- heterozygous mice exhibited no apparent phenotypic abnormalities during development and adulthood.

Heterozygous VGLUT1 and WT male mice (C57BL/6) (8-10 weeks of age) were housed in individual cages and allowed for 2 weeks to habituate before beginning experimentation. Food and water were available *ad libitum* for the duration of the experiments unless otherwise specified. Animals were maintained in a temperature (21 $\pm$ 1 ºC) and humidity-controlled room (55 $\pm$ 2 %) on a 12 h light-dark cycle (lights on at 08:00 h).

Experimental procedures and animal husbandry were conducted according to the principles of laboratory animal care as detailed in the European Communities Council Directive (2003/65/EC), Spanish legislation (Real decreto 1201/2005) and approved by the Ethical Committee of University of Navarra.

2.2 Experimental design

Both WT and VGLUT1+/- mice were divided into control and CMS groups (n=15mice/group, four groups, n=60 in total). Chronic mild stress (CMS) procedure was applied for six weeks (Elizalde et al., 2008). Anhedonic-like behavior was evaluated by weekly monitoring of sucrose intake in both control and CMS mice during the 6 weeks of the stress procedure. Over the last week of CMS, a battery of behavioural tests including motor activity, novel object recognition, test for anxiety (elevated plus maze) and depression (forced swimming test) were performed. The behavioural phenotype of these groups have been previously shown (García-García et al., 2009). Tests were performed from 9:00-1:00 p.m. Animals were sacrificed 76 h after the last test (forced swimming test) and 24 h after the last stress procedure. Mice were killed by cervical dislocation, brains were rapidly removed and the prefrontal cortex (around 15 mg) was rapidly dissected according to standard procedures and frozen in dry ice. Samples were stored at -80°C until RNA was isolated.

2.3 Chronic mild stress procedure

The following unpredictable mild stressors (2-3 in any 24 h period) were randomly applied for 6 weeks (Elizalde et al., 2008): stroboscopic illumination (8h), intermittent bell (10 db, 1s/10s) or white noise (4 h), rat odour (8h), cage tilt 45º (8 h), soiled bedding (6 h), paired housing (2h) overnight illumination, removal of nesting material (12 h) and confinement (1 h). Once a week, anhedonic-like behaviour was evaluated by weekly monitoring of sucrose intake (Elizalde et al., 2008). During the 15 h of duration of the sucrose intake test (from 6 p.m to 9 a.m.) no stressors were applied.

2.4 Sucrose intake test

Anhedonic like behaviour was evaluated by weekly monitoring of sucrose intake (Elizalde et al., 2008). Mice were first trained to drink a sucrose solution by exposing them to two standard drinking bottles, one containing 2.5 % sucrose and the other tap water, for every other night during one week. After this preliminary phase, mice were food deprived and exposed to the sucrose solution and water from 6:00 p.m. until 09:00 h in the morning, once a week during 6 weeks. The intake baseline for the sucrose solution was established, which corresponded to the average of three consecutive measurements.

WT and VGLUT1+/- mice were subdivided into two groups (CMS and non-stressed controls) matched for sucrose consumption and body weight. Mice were weekly given a 15-h exposure to the sucrose solution and tap water as described above and during this test no stressors were applied. The position of the 2 bottles (right/left) was varied randomly from trial to trial. Body weight measurements were taken weekly and relative sucrose intake was calculated as absolute intake (g) per body weight.

2.5 Forced swimming test

Mice were individually placed into glass cylinders (height 24 cm, diameter 13 cm) containing water (14 cm, 22–23°C). Immobility, indicative of helpless behaviour, was recorded during the last 4 min of the 6 min testing period and is considered a measure of helpless behaviour.

2.6 RNA extraction

Isolation of total RNA was carried out according to manufacturer's instructions (NucleoSpin RNA II kit, Macherey-Nagel). Total RNA was isolated separately from each individual cortex. The frozen prefrontal cortex samples were lysed and dounze-homogenized in the presence of a highly denaturing ß-mercaptoethanol-containing buffer, which immediately inactivates RNases. Ethanol was added to provide appropriate binding conditions, and the sample was then applied to an RNeasy Mini spin column, where the total RNA binded to the membrane and contaminants were washed away. RNA was then eluted in 30–60 µL RNase-free water. The eluates were stored at –80°C.

2.7 Microarray hybridization

RNA quality control was checked with the Agilent Bioanalyzer (Agilent Technologies Inc, Santa Clara, CA, USA) and the RNA concentration was evaluated by using a NanoDrop™ Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Microarray hybridizations were outsourced to ServiceXS B.V. (BZ Leiden, The Netherlands). Nine animals out of 15 in each group (control and CMS, WT and VGLUT1+/- mice) were used for large-scale gene expression analysis. Selection of the animals was based on the RNA quality from the frontal cortex extracts. RNA from groups of three animals was pooled to reduce noise and individual variability, giving three samples per group, which were treated as such for statistical analysis. The Illumina® TotalPrep™ RNA Amplification Kit (Ambion Inc, Austin, TX, USA) was used to synthesize biotine labeled cRNA. Then, 750 ng of biotin labeled cRNA were

hybridized onto the MouseRef-8 v2.0 Expression BeadChips (Illumina Inc., San Diedo, CA, USA) accordingly to manufacturer's instructions. The used multi-sample array format allows to assay approximately 25,600 transcripts and to profile eight samples simultaneously on a single MouseRef-8 v2.0 Expression BeadChip.

2.8 Validation of the differentially expressed genes by RT-PCR

TaqMan Low Density Arrays (TLDA) microfluidic card technology from Applied Biosystems (Foster City, CA, USA) was used to validate the differential expression of selected genes (Table 1) in 6 independent samples per group. Each reaction well contained all reagents specific for a given assay. For each tissue sample, 100 ng reverse-transcribed RNA were diluted to 50 µL with sterile water, combined with an equal volume of TaqMan Universal PCR Master Mix (2×; Applied Biosystems, Foster City, CA, USA), mixed by inversion, and spun briefly in an Eppendorf® 5415C microcentrifuge (Brinkmann Instruments, Westbury, NY, USA). After TLDAs were brought to room temperature, 100 µL master mix were loaded into each port connected to reaction wells. TLDAs were placed in Sorvall®/Heraeus® Custom Buckets (Applied Biosystems, Foster City, CA, USA) and centrifuged in a Sorvall Legend™ centrifuge (Kendro Scientific, Asheville, NC, USA) for 1 min at 331×g followed closely by a second 1-min centrifugation at 331 g. Cards with excess sample in the fill reservoir were spun for an additional 1 min. Immediately following centrifugation, the cards were sealed with a TaqMan LDA Stylus Staker (Applied Biosystems, Foster City, CA, USA), and the loading ports excised. The final volume in each well after centrifugation was <1.5 µL; thus, the final reverse-transcribed RNA concentration was approximately 1.5 ng/reaction. Quantitative RT-PCR amplifications were run on an ABI Prism® 7900HT Sequence Detection System (Applied

Biosystems, Foster City, CA, USA) with a TaqMan LDA cycling block and an automation accessory upgrade. Thermal cycling conditions were 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min. Each test sample was processed in duplicate on individual TLDA cards, thus allowing four samples to be processed on each card.

2.9 Analysis of quantitative RT-PCR Data

RT-PCR TaqMan instrumentation monitors gene-specific products with fluorescent dye chemistry. A cycle threshold (CT) for each reaction is the number of cycles at which the reaction crosses a selected threshold. The threshold is defined as a straight line drawn above noise/baseline and positioned within the linear region of the semi-log amplification plot. The fewer cycles required to reach threshold fluorescence intensity, the lower the CT value and the greater the initial amount of input target. Results for each target on TLDAs were quantified concurrently using the same baseline and threshold for a target gene in order to limit interplate errors in the analysis.

Samples were analyzed by the double delta CT (ΔΔCT) method. Delta CT (ΔCT) values represent normalized target genes levels with respect the internal control. Normalization was based on a single reference gene (GAPDH). Delta CT (ΔΔCT) values were calculated as the ΔCT of each test sample (stressed wild-type or non-stressed/stressed CB_1 knockout mice) minus the mean ΔCT of the calibrator samples (non-stressed wild-type) for each target gene. The fold change was calculated using the equation $2^{(-\Delta\Delta CT)}$.

2.10 Data normalization and analysis

Behavioural studies. Immobility in the forced swimming test was analyzed using Student t-test analysis. For the sucrose intake test one way ANOVA repeated measures followed by Student t-test for individual weeks was applied.

Gene expression studies. Data normalization and gene expression analysis was carried out with the R/Bioconductor package beadarray (Dunning et al., 2007). The data normalization was performed by using the quantile normalization algorithm (Bolstad et al., 2003). The statistical analysis were based on the Propagating Uncertainty in Microarray Analysis (PUMA) method that allows to identify differentially expressed genes by combining biological replicates and to perform a principal component analysis (PCA) (Rattray et al, 2006). The filtering criterion used to define statistically significant differentially expressed genes was the minimum Pplr (MinPplr) lower than 0.001.

Functional enrichment analysis of Gene Ontology (GO) categories (http://www.geneontology.org/) and KEGG pathways (http://www.genome.ad.jp/kegg/) was carried out using standard hypergeometric test (Huang et al, 2009). To identify biologically relevant clusters of interrelated genes a stringency criterion of $p<0.01$ were adopted in DAVID software (Huang et al, 2009).

The functional interpretation and biological knowledge extraction was complemented through the use of Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com), which database includes manually curated and fully traceable data derived from literature sources. Additionally, the lists of differentially expressed

genes were browsed to find genes encoding neurotransmitter metabolizing enzymes, neurotransmitter receptors or receptor subunits, neurotrophic factors and others genes related to nervous system.

3. Results

3.1. Forced swimming and sucrose intake tests

Mice body weight was not affected neither by CMS or genotype. CMS mice decreased sucrose intake (solution (g)/body weight) from the fourth week of CMS until the end of this procedure (Fig. 1A). VGLUT1+/- mice decreased also sucrose intake measured as the average of sucrose intake on three consecutive weeks (Fig. 1C).

In the forced swimming test (FST), increased immobility times were observed in CMS exposed mice (Fig. 1B). On the other hand, VGLUT1+/- heterozygous mice showed a significant increase in the immobility time in the FST compared to their WT littermates (Fig. 1D).

3.2 Patterns of altered gene expression in VGLUT1+/- mice compared to WT

We considered statistical significance levels of MinPplr<0.001 when comparing patterns of gene expression on microarray among the different groups. In addition, significant levels of MinPplr<0.005 were also considered when differences in gene expression levels were higher than 1.4 or lower than 0.7 times of WT control levels. The levels of 1046 gene transcripts were significantly affected by genotype in the prefrontal cortex at basal conditions being 583 dowregulated and 463 upregulated (see clustering analysis results in supplemental material, figure 1s).

Following the The DAVID 2008 Functional Annotation Tool was used to identify enriched biological pathways in the lists of differentially expressed genes obtained according to KEGG pathways and GO categories. After this functional analysis a set

of enriched KEGG pathways including apoptosis, neurogenesis, synaptic transmission, protein metabolic process or learning and memory were detected (summarized in Table 2).

From all the significant genes, we selected some candidates to validate considering their relationship with different neurotransmitter systems and the coincidence with the results obtained by analyzing differential gene pattern in another animal model of depression, the CB1 KO mice (Aso et al., 2009). In total, the gene expression of 20 transcripts was evaluated by quantitative RT-PCR (Table1). Among them, RT-PCR confirmed the differential expression of several genes involved in GABA and glutamate transmission, including a dowregulation of VGLUT1, ionotropic kainate glutamate receptor 5 (**Grik5**) and an upregulation of GABA-A receptor subunits (**Gabrg2** and **Gabra3**), GABA-B receptor subtype 1 (**Gabbr1**), glycine receptor beta subunit (**Glrb**), member of the Ras oncogene family 8 (**Rab8b**) and member A of the ras homolog gene family (**Rhoa**). In addition, a dowregulation of the dopamine receptor 1a (**Drd1a**) and an upregulation of the 5-hydroxytryptamine receptor 1A (**Htr1a**) and adenilate cyclase 8 (**Adyc8**) were also confirmed (Table 1).

3.3 Patterns of altered gene expressions common to the chronic mild stress and VGLUT1+/- model

Similarly, 147 transcripts showed significantly altered expression levels after exposure to CMS; 94 were downregulated and 41 were upregulated. (See clustering analysis results in supplemental material, figure 2s). CMS affected the regulation of 147 transcripts, some of them involved in response to stress and oxidoreductase activity. We identified 52 genes similarly regulated by both the CMS and the

VGLUT1+/- models being 37 downregulated and 15 upregulated. Among them, genes playing an important role on cell proliferation and survival, apoptosis, oxidative metabolism, glutamate transmission or cytoskeleton was included (Table 3, and see hierarchical clustering image in Figure 2). Specifically, a dowregulation in genes that promote cell proliferation (**Anapc7**; anaphase promoting complex subunit 7), cell growth (**CsnK1g1;** casein kinase 1 gamma 1), cell survival (**Hdac3**; histone deacetylase 3), inhibition of apoptosis (**Dido1;** death induced-obliterator 1) was observed. Other genes linked to cytoskeleton (**Hspg2**; perlecan, **Invs;** inversin), psychiatric disorders (**Grin1**; glutamate receptor, ionotropic, **MapK12**; mitogen-activated protein kinase 12) or an antioxidant enzyme (**Gpx2**; glutathione peroxidase 2) were also downregulated. In addition, an upregulation of genes that inhibit the MAPK pathways (**Dusp14;** dual specificity phosphatase 14), stimulate oxidative metabolism (**Eif4a2;** eukaryotic translation initiation factor 4A2) and enhance glutamate transmission (**Rab8b**; member RAS oncogene family 8) was detected. On the other hand, genes supposed to be neuroprotective such as an inhibitor of apoptosis (**Pip5k1a**; phosphatidylinositol-4-phosphate 5-kinase, type 1 alpha), an stimulator of cell differentiation (**Nedd9**; neural precursor cell expressed developmentally down-regulated gene 9) as well as genes linked to antioxidant activity (**Prnp;** prion protein) or cytoskeleton (**Catna1;** catenin alpha 1) were also upregulated.

4. Discussion

Here, we firstly show that in agreement with previous studies carried out in our laboratory (García-García et al., 2009; Elizalde et al., 2008) both the genetic (VGLUT1+/- mice) and the environmental (chronic mild stress) model of depression showed helpless and anhedonic-like behavior.

The vesicular glutamate transporter 1 (VGLUT1) is the major isoform in cortical and hippocampal regions (Takamori et al., 2000; Fremeau et al., 2001), where it plays a key role in the vesicular uptake and synaptic transmission of glutamate (Wojcik et al., 2004: Fremeau et al., 2004; Balschun et al., 2009) in telencephalic areas. Thus, the anhedonic and helpless behaviour shown by these mice could be linked to decreased glutamate transmission in those areas in which VGLUT1 is the major isoform. In keeping with this, recent post mortem studies showing decreased cortical VGLUT1 in depressed subjects (Uezato et al., 2009) together with clinical findings of an excitatory inhibitory imbalance in the cortex of depressed patients (Sanacora et al., 2004; Bhagwagar et al., 2007) suggest that decreased VGLUT1 levels might have functional and clinical implications.

Microarray studies in VGLUT1+/- mice revealed regulation of genes involved in apoptosis, neurogenesis, synaptic transmission, protein metabolic process or learning and memory. Importantly, given that glutamate transmission is reduced in both VGLUT1+/- (Balschun et al., 2009) and VGLUT2+/- mice (Moechars et al., 2006) and that both complementary isoforms show identical pharmacological properties (Takamori et al., 2001) it would be interesting to identify which of these differentially expressed genes are common in both genetic models. These studies would add relevant information like which gene changes are directly linked to

impaired glutamate transmission and which could be more related to developmental adaptations within a particular brain area.

We selected some candidates to validate by RT-PCR considering their relationship with different neurotransmitter systems and the coincidence with the results obtained by analyzing differential gene pattern in another animal model of depression, the CB1 KO mice (Aso et al., In this Issue). Changes in several glutamate, GABA, dopamine and serotonin neurotransmitter receptors were confirmed. Interestingly, VGLUT1+/- mice showed an upregulation of GABA-A receptor subunits (**Gabrg2** and **Gabra3**), GABA-B receptor subtype 1 (**Gabbr1**), glycine receptor beta subunit (**Glrb**) and a downregulation of the ionotropic kainate glutamate receptor 5 (**Grik5**). Some of these changes might have a clinical relevance for different psychiatric disorders. For instance, associations between a polymorphism in GRIK5 receptor subunit (Gratacos et al., 2009) and an upregulation of the Gabra3 (Massat et al., 2002) to bipolar disorder have been described.

We suggest that the upregulation of the different GABA subunits and receptors could be a compensatory effect for decreased GABA levels in the synaptic cleft. Indeed, previous studies in our laboratory show that VGLUT1 heterozygous mice exhibit normal levels of glutamate but low cortical and hippocampal levels of GABA without alterations of the GAD65 synthesizing enzyme (García-García et al., 2009). Moreover, these mice show a downregulation of the excitatory aminoacid transpoter 1 (EAAT1) (García-García et al., 2009). It has been suggested that a downregulation of EAAT1 could limit the glial glutamate uptake from the synaptic cleft and limit the synthesis of GABA in the GABAergic neuron (Mathews and Diamond, 2003).

Nowadays, there is growing evidence confirming the implication of the GABAergic dysfunction (Krystal et al., 2002; Brambilla et al., 2003; Tunnicliff and Malatynska.,

2003). Clinical studies have shown that depressed patients have reduced GABA levels in cortex demonstrated by proton magnetic resonance spectroscopy (MRS) (Sanacora et al., 1999, 2004), plasma (Tunnicliff and Malatynska., 2003) and cerebrospinal fluid. Moreover, preclinical studies show decreased GABA levels in animal models of depression (Brambilla et al., 2003; Gronli et al., 2007; Sanacora et al., 2007; Garcia-Garcia et al., 2009).

On the other hand, the upregulation of both **Rhoa** and **Rab8b** could be also a compensatory effect for a decreased glutamate release in the VGLUT1 dependent glutamatergic terminals. Both proteins are involved in the glutamate synaptic streght and in the release of AMPA receptors to the dendritic spines (Gerges et al., 2004). Further studies should investigate whether AMPA transmission is affected in these mice. Taken together, the RT-PCR studies further support the idea of an altered balance between the excitatory (glutamate) and inhibitory (GABA) transmission in these mice.

Among the rest of neurotransmitter receptors, a dowregulation of the dopamine receptor 1a (**Drd1a**) agree with the CB1 KO model (Aso et al., 2010, in this special issue).

The chronic mild stress model has been extensively studied at the behavioural (Strekalova et al., 2004;2006) molecular (Airan et al., 2007; Gronli et al., 2007; Banasr et al., 2008; García-García et al., 2009) or cellular (Warner-Schmidt and Duman 2006; Jayatissa et al., 2008) levels. In addition to core symptoms of depression, such as long-lasting anhedonia (Elizalde et al., 2008), CMS induces neuroadaptive changes that could be addressing clinical findings with depressed patients (Gould et al., 2007; Sanacora et al., 2004; Rajkowska., 2000; Lucassen et

al., 2006; Frodl et al., 2008). CMS affected the regulation of 147 transcripts, some of them involved in response to stress and oxidoreductase activity. Here, we aimed to identify in these two experimental models (CMS and VGLUT1+/- mice), gene expression changes common to stress and impaired glutamate function.

Specifically, a dowregulation in genes that promote cell proliferation (**Anapc7**) (Gieffers et al., 2001), cell growth (**CsnK1g1**) (Kusuda et al., 2000), cell survival (**Hdac3**) (Xia et al., 2007), inhibition of apoptosis (**Dido1**) (Fütterer et al., 2005) was observed. Genes linked to cytoskeleton (**Hspg2**, **Invs**) (Farach-Carson et al., 2008; Nürnberger et al., 2004), psychiatric disorders (**Grin1**, **MapK12**) (Georgi et al., 2007; Qi et al., 2006) or an antioxidant enzyme (**Gpx2**) (Ranjekar et al., 2003) were also downregulated. Moreover, genes that inhibit the MAPK pathways (**Dusp14**), (Kingler et al., 2008) stimulate oxidative metabolism (**Eif4a2**) (Cheyssac et al., 2008) and enhance glutamate transmission (**Rab8b**) (Gerges et al., 2004) were upregulated. Interestingly these changes agree different hypothesis that link major depression to either structural changes such as an increased neuronal death, neurite atrophia or molecular changes that lead to a failure in synaptic function and plasticity (Duman et al., 2006). Yet, the functional relevance of these changes for major depression would be further supported by gene expression studies on other genetic models.

On the other hand, perhaps as compensatory mechanims, genes supposed to be neuroprotective such as an inhibitor of apoptosis (**Pip5k1a**; phosphatidylinositol-4phosphate 5-kinase, type 1 alpha), (Bassi et al., 2008) an stimulator of cell differentiation (**Nedd9**; neural precursor cell expressed developmentally down-regulated gene 9) (Sasaki et al., 2005) as well as genes linked to antioxidant activity (**Prnp;** prion protein) (Rachidi et al., 2003) or cytoskeleton (**Catna1;** catenin alpha 1) (Park et al., 2002) were upregulated.

Summing up, we have identified here genes similarly regulated by both environmental (CMS) and genetic model (VGLUT1+/- heterozygous mice) that could form part of the altered “molecular context” underlying depressive-like behaviour in these models and might provide new insights into the molecular basis of clinical depression.

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Figure legends

Figure 1. Performance of WT-CMS and WT control mice in the sucrose intake (A) and in the forced swimming test (B). In addition, performance of VGLUT1 heterozygous and wild type (WT) mice on the sucrose intake test (C) and on the forced swimming test (D). Values show the mean ± SEM (n=15 mice/group). In VGLUT 1+/- mice, the average of the sucrose intake of three consecutive weeks was calculated. *p<0.05 *vs* corresponding control or WT mice (One-way ANOVA repeated measures for the sucrose intake test during CMS, and Student *t*-test for the rest of the experiments).

Figure 2. Hierarchical clustering analysis of gene expression profiles shows similar patterns of differentially expressed transcripts in the prefrontal cortex for WT mice exposed to CMS and VGLUT1+/- mice.

Figure 1S. Hierarchical cluster analysis of expression profiles corresponding to the differentially expressed transcripts in the prefrontal cortex of VGLUT1+/- compared to WT mice.

Figure 2S. Hierarchical cluster analysis of expression profiles corresponding to the differentially expressed transcripts in the prefrontal cortex of WT mice exposed to CMS compared to WT controls.

Author Disclosure

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Contributors

Dr Rosa M. Tordera, Natalia Elizalde and Alvaro García-García performed the behavioural experiments and RNA extractions. In addition, Dr Rosa M. Tordera carried out the data collection analysis of gene expression and wrote the manuscript first draft. Dr Ester Asó carried out the quantitative RT-PCR experiments. Dr Victor Segura contributed to the microarray data interpretation. Elisabet Venzala carried out the genotyping. Dr Maria J. Ramírez contributed to the microarray data discussion and interpretation. Prof. Joaquin Del Rio oversaw the project and contributed to the final draft of the paper. All authors contributed and agreed to the final draft of the paper.

Conflict of interest

All the authors declare no conflict of interest.