



Shared changes in gene expression in frontal cortex of four genetically modified mouse models of depression

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Received 14 April 2010; received in revised form 21 September 2010; accepted 24 September 2010

KEYWORDS

Depression;
Gene expression;
Cellular stress;
Remodelling

Abstract

This study aimed to identify whether genetic manipulation of four systems implicated in the pathogenesis of depression converge on shared molecular processes underpinning depression-like behaviour in mice. Altered 5HT function was modelled using the 5-HT transporter knock out mouse, impaired glucocorticoid receptor (GR) function using an antisense-induced knock down mouse, disrupted glutamate function using a heterozygous KO of the vesicular glutamate transporter 1 gene, and impaired cannabinoid signalling using the cannabinoid 1 receptor KO mouse. All 4 four genetically modified mice were previously shown to show exaggerated helpless behaviour compared to wild-type controls and variable degrees of anxiety and anhedonic behaviour. mRNA was extracted from frontal cortex and hybridised to Illumina microarrays. Combined contrast analysis was used to identify genes showing different patterns of up- and down-regulation across the 4 models. 1823 genes were differentially regulated. They were over-represented in gene ontology categories of metabolism, protein handling and synapse. In each model compared to wild-type mice of the same genetic background, a number of genes showed increased expression changes of >10%, other genes showed decreases in each model. Most of the

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genes showed mixed effects. Several previous array findings were replicated. The results point to cellular stress and changes in post-synaptic remodelling as final common mechanisms of depression and resilience.

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1. Introduction

1.1. Overview

In the search for new molecular clues to the pathogenesis of depression, a number of gene array studies in human post-mortem brain and in rodent models of depression have been carried out. Many different findings have been reported but some possible commonalities can be discerned and they are summarised below. This study describes an attempt to find commonalities in patterns of gene expression in frontal cortex from 4 behaviourally validated mouse models each with a different genetic modification (GM) of a known vulnerability mechanism. The changes are compared with findings from the previous literature.

1.2. Genes and pathways implicated in major depressive disorder (MDD) — human post-mortem brain array studies

GABA and glutamate neurotransmission-related genes emerged most frequently from array studies with human post-mortem brain from MDD patients who committed suicide (Choudary et al., 2005; Kim and Webster, 2008; Klempan et al., 2009b; Sequeira et al., 2007, 2009). Interestingly neither serotonergic, nor adrenergic genes showed alteration in the array studies so far (Sequeira et al., 2009). In addition several astrocyte/oligodendroglia-related genes were down-regulated in these patients (Choudary et al., 2005; Klempan et al., 2009b,a; Sibille et al., 2009; Sokolov, 2007). Other pathways implicated by array studies in MDD include: central nervous system (CNS) development, cell proliferation/pathfinding, programmed cell death and the ubiquitin-mediated proteolysis pathway (Altar et al., 2009; Kang et al., 2007; Kim and Webster, 2008; Tochigi et al., 2008). Differential expression of genes related to chemokine signalling, interferon system and tumor necrosis factor in MDD cases suggests the involvement of inflammatory mechanisms (Kang et al., 2007; Lalovic et al., 2010).

1.3. Candidate genes and pathways from brain array studies in rodent stress models of depression

Changes in GABA and glutamate-related gene expression have been reported in hippocampus and hypothalamus of rats subjected to the early maternal separation paradigm (van Heerden et al., 2009), whereas others report changes in apoptosis, metabolism, signal transduction, cell–cell interaction and development (Liebl et al., 2009), and in the brain renin–angiotensin system (Kohda et al., 2006; Liebl et al., 2009) in this model. Chronic mild stress-induced anhedonia has been associated with alteration in pathways of energy

metabolism, signal transduction, transcription, synaptic plasticity, and remodelling of the brain architecture (Kim and Han, 2006). Antidepressant treatment caused opposite changes in neuroplasticity and neurogenesis pathways which paralleled the decrease of symptoms (Bergstrom et al., 2007; Orsetti et al., 2009; Sillaber et al., 2008; Surget et al., 2009). Models based on administering steroids (e.g. prednisolone, dexamethasone, corticosterone) showed effects most prominently on cell-death related pathways in the hippocampus (Kajiyama et al., 2010) but also in many hypothalamic genes with and without glucocorticoid response elements (Sato et al., 2008). Cerebral cortex showed similar extended alteration in various transcription pathways, neuron differentiation and apoptosis (Uriguen et al., 2008).

1.4. GM mouse models and study design

In the present study, we compared 4 GM mouse models of depression taking as our starting point genes in four systems most directly implicated in human studies of depression: 5-hydroxytryptamine (5-HT), the hypothalamo-pituitary-adrenal (HPA) system, glutamate and cannabinoid signalling. Altered 5-HT function and impaired negative feedback control of the HPA have been described in depression for several decades (Belmaker and Agam, 2008). More recent interest has focussed on glutamate and cannabinoid systems as candidate mechanisms for depression, largely on the basis of antidepressant drug effects and the ability of the cannabinoid receptor antagonist rimonabant to precipitate depressive illness (Christensen et al., 2007).

The mouse models were selected according to the pre-determined behavioural criteria and harmonised procedures of the NewMood project — that they show learned immobility in the tail suspension or forced swim tests, increased anxiety-like behaviour in the light–dark emergence or open field tests and anhedonia in the sucrose preference test. The four models selected comprised three knock-outs; 5-HT transporter (HTT^{-/-}; (Bengel et al., 1998)), vesicular glutamate transporter (VGLUT^{-/+}; (Garcia-Garcia et al., 2009)), cannabinoid 1 receptor (CNR1^{-/-}; (Martin et al., 2002)) and an antisense knock down of the glucocorticoid receptor (GRi; (Lanfumeijer et al., 2000)). A uniform method of brain dissection and mRNA extraction was used. Quality control and hybridisation was carried out by a service company using Illumina arrays.

The probability of the same gene appearing in separate gene lists from different models decreases as the number of models increases. Indeed, in individual analyses of the present 4 models with false discovery rate (FDR) threshold of 0.01, a total of 889 genes were differentially expressed but there were no co-occurrences in 3 or in all 4 models; 23 genes appeared in pairs of models. Because of thresholding, the significance of systematic sub-threshold changes is not assessed in the gene list approach. Furthermore, a gene

showing an adaptive up-regulation in one model could show down-regulation in another and contribute to the phenotype. Therefore, we used a combined contrasts method to detect genes showing any combination of increases, decreases and no change in expression across the GM models. In addition, a hypothesis-testing replication analysis was carried out on gene candidates identified in the array studies reviewed above.

2. Method

2.1. Genetically modified mice

CNR1^{-/-}: The generation of mice lacking CB1 cannabinoid receptor on a CD1 background is described in [Martin et al. \(2002\)](#). HTT^{-/-}: Homozygous male HTT KO and 5-HTT WT littermates born from heterozygous mutants of C57BL/6J genetic background ([Bengel et al., 1998](#)). GR1: Glucocorticoid receptor antisense mice (Gr-i) mice were bred on a F1 B6/C3H line ([Froger et al., 2004](#)). VGLUT1^{+/-}: male mice (VGLUT1^{+/-}; C57BL/6) (8–10 weeks of age) from S. Wojcik (Göttingen, Germany) were used. A colony of wild-type (WT) and VGLUT1^{+/-} mice were bred from heterozygous fathers and WT mothers (Harlan, France). None of the GM mice experienced chronic stress procedures. 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).

3. Microarray experiments

Microarray experiments were conducted as part as the EC Framework 6 Integrated Project NewMood and the procedures (selection of the brain region, dissection, sample preparation, hybridization, bioinformatics) were agreed upon by the partners of the consortium.

3.1. RNA extraction

Male mice (~2 months-old) of each genotype were sacrificed by cervical dislocation between 10:00 and 12:00 am in a counterbalanced order and the brain was removed and dissected. Regions were frozen in liquid nitrogen and stored at -80 °C until use. Total RNA was extracted using the NucleoSpin RNA II extraction kit (Macherey-Nagel, Germany), including removal of genomic DNA by DNAase treatment. RNA integrity was checked by agarose gel electrophoresis, and RNA concentrations were determined using micro-spectrophotometry (NanoDrop Technologies, Houston, TX, USA). For 3 of the GM models, 12 animals from each group were sacrificed. RNA from three animals was pooled based on the individual RNA concentrations to provide 4 pools per group for hybridisation. Pooling took place either in the partner's own labs (CB1 KO) or at ServiceXS. In the case of VGLUT1 mice 3 pools of 3 per group were created. RNA content was re-assayed and then hybridised to the BeadChips and scanned.

The gene expression studies followed a standard protocol by ServiceXS (www.servicexs.com/) using the Illumina MouseRef-8 Expression BeadChip, which has more than 24,000 transcript-specific probes/array. The probes (50-mers covalently coupled to 3-micron beads) are derived from the Mouse Exonic Evidence Based Oligonucleotide (MEEBO)

set as well as exemplar protein-coding sequences described in the Riken Fantom2 database. The raw data were collected and the files made available (password controlled) to the analysis team (at the University of Manchester) who normalised the data and sent it to the relevant partners.

3.2. Statistical analysis

All statistical analyses of the expression data were carried out within the R statistical software environment [www.r-project.org]. The logarithm of the raw intensity values was taken and a quantile normalization was applied to the data from each individual mouse model, following the procedure used by ([Altoa et al., 2010](#)). For the combined analysis across all four mouse models the initial normalized data from each mouse was re-centred to a common value. Principal Component Analysis (PCA) ([Jolliffe, 1986](#)) of the combined normalized data was then performed that revealed a second principal component that correlated with the date on which the RNA hybridizations were performed. The contribution from the second principal component was therefore removed and the data re-normalized by applying a median polish (median row and column centering) ([Tukey, 1977](#)). A linear model was used to detect probes displaying statistically significant differences in gene expression between the wild-type and GM animals using limma Bioconductor package ([Smyth, 2005](#)) within R. No probes showing differential GM/WT expression across all 4 models survived FDR ≤ 0.01 correction ([Benjamini and Hochberg, 1995](#)). The combined contrast analysis examined the log fold changes across models for deviations from chance as a more suitable method for ranking and selecting the probes. This analysis tests the null hypothesis that a probe is not differentially expressed in any of the four mouse models. Rejection of the null hypothesis effectively selects probes that are differentially expressed in at least one of the mouse models. Further analyses (below) were performed to identify the biological trends within the data and to mitigate the effects of probes that display a statistically significant combined contrast primarily due to a large fold-change in just one of the mouse models.

Probe lists identified from the combined contrast analysis were subjected to analysis for statistically significant enrichment in GO categories using the DAVID bioinformatics software tool ([Huang et al., 2009](#)). Genes corresponding to probes that showed more than 10% changes in the same direction across all 4 models ('all up' or 'all down') and 20% up or down-regulation in each model ('mixed') were also entered into searches (principally PubMed, entrez-gene and genecard) for further information and reported association with depression in human and animal array studies and in human genetic studies.

To test for replication of previous array results (hypothesis-testing analysis), we searched PubMed in January 2010 for whole-genome array studies using queries of a i) gene expression and depression, ii) gene expression and maternal deprivation, iii) gene expression and chronic mild/unpredictable stress. 2759 candidate genes were collated and those showing combined contrast effects across the 4 GM models were identified using the same multiple testing correction procedure as the whole-genome analysis but based on the smaller number of tests.

4. Results

4.1. Combined contrast analysis: functional categories

1823 genes showed differential patterns of expression across the 4 models. Functional analysis by GO category revealed significant enrichment (Benjamini corrected $p < .05$) in a number of GO categories listed in Table 1. They include categories to do with: cellular metabolism including mitochondrial genes; protein processing including ribosomal genes, protein localisation and the ubiquitin pathway; vesicular transport; and synapse. Of the mitochondrial genes, 7 were up-regulated in all models and 2 down-regulated but none showed changes of greater than the 10% reporting threshold in all models. The rest showed mixed up- and down-regulation with the *Was2* mitochondrial gene showing greater than 20% change in all models (Table 2c). Of the ribosomal genes, none were down-regulated in all 4 models. In the VGLUT1 model all ribosomal genes except one showed fold changes of greater than unity (mean 1.40, SD.27) whereas the other models had mean fold changes of 1.00 with SDs of less than 0.13.

The synaptic genes included pre- and post-synaptic elements. In the post-synaptic proteins, the most striking deviations were seen for the gene encoding activity-related cytoskeletal-associated *Arc* protein which was down-regulated by 42% in the CB1 model and up-regulated by 21–23% in the other three models (Table 3). In many other cases the combined contrast was driven by the greater deviations seen in the VGLUT1 model. For example, increased gene expression of GABA-A subunits and decreases in glutamate receptor gene expression were largely confined to the VGLUT1 model (Table 3). Similarly, changes in presynaptic genes were most marked in the VGLUT1 model although changes in synaptic vesicle protein 2a (*Sv2a*) showed deviations of 14–36% in all models.

Table 1 Gene Ontology (DAVID) analysis of 1625 genes showing differential expression across GM models. FDR E = decimal places < 5.0 in Benjamini false discovery rate (i.e. all $< .05$).

DAVID GO Category	Enrichment	FDR E	Genes
Intracellular	1.27	−30	927
Metabolic	1.21	−9	675
Protein localisation	1.74	−6	109
Intracellular organelle	1.30	−5	275
Ribosome	2.51	−5	40
Mitochondrion	1.62	−4	90
Vesicle mediated transport	1.86	−4	65
Negative regulation of cellular process	1.47	−3	109
Catabolic process	1.58	−3	75
Cellular carbohydrate metabolic process	1.97	−3	43
Cellular respiration	4.57	−3	12
Transcription co-factor activity	2.35	−2	29
Ubiquitin–protein ligase activity	2.54	−2	26
Synapse	1.86	−2	36

4.2. Top genes across models

121 genes showed increased expression to varying degrees across the four models of which 5 showed fold changes of more than 10% in all models. Ninety genes showed varying decreases across all 4 models of which 2 showed decreases of at least 10%. Genes showing more than 10% changes in all models are listed in Table 2a and b. 1614 genes showed mixed increases and decreases across models of which 8 showed changes of 20% or greater in each model. The complete lists are available in Supplementary material.

4.3. Hypothesis-testing replication analysis

238 of the 2759 genes previously reported in array studies, including 42 genes from studies in human post-mortem brain in depression (most in frontal cortex), were differentially expressed across the 4 models but they all also appeared in the hypothesis-free list of 1823 genes. 22 showed changes of greater than 10% in all models including 2 genes from human brain studies (Supplementary materials).

5. Discussion

5.1. Differentially up-regulated genes

Erythrocyte differentiation regulator 1 (*Erd1*) was variably up-regulated in all 4 GM mouse models. This little-known gene encodes a protein that induces the development of erythrocytes from haemopoietic tissue. However, a previous study found *Erd1* was one of 8 genes whose expression was strongly inversely related to behavioural measures of anxiety in 6 strains of mouse in hippocampus and hypothalamus (Hovatta et al., 2005). In the present study it was variably up-regulated in all 4 GM mouse models all of which show behavioural evidence of increased anxiety-like behaviour using similar methods to Hovatta et al. The direction of change in expression is opposite to that previously reported in relation to the anxiety phenotype. Another 4 of the 17 genes from Hovatta et al. were differentially expressed in the 4 GM models (Supplementary material). All 4 were positively correlated with anxiety in Hovatta et al. and local lentivirus induced over-expression of *Glo1* (glyoxalase I) and *Gsr* (glutathione reductase) in brain increased anxiety-like behaviour. *Glo1* was increased by 36% in VGLUT1 model and *Gsr* by 40% in the HTT model suggesting these genes may have a causal role in anxiety in these models. However *Gsr* was reduced by 45% in the CNR1 model and this could represent an adaptive response to a different molecular pathway leading to anxiety/depression.

Hovatta et al. pointed out that several of their anxiety-related genes are involved in oxidative metabolism. Peroxisomes have an important role in oxidative stress but the 7 differentially expressed peroxisomal genes in the GM models did not amount to significant over-represented in the David peroxisome category. Ubiquitination is important for protein transport into peroxisomes and genes from the mixed contrast list were over-represented in this category (Table 1) with different genes up- and down-regulated in each of the models. These patterns raise the possibility that the GM models involve cellular stress. In keeping with this idea, the up-regulated serum/glucocorticoid regulated kinase (*Sgk*) gene (Table 2a) is

Table 2 Genes showing suprathreshold changes in all 4 GM models.

Symbol	Definition	CNR1	VGLUT1	HTT	GRI
<i>a) Genes showing >10% increases in each model</i>					
<i>Erd1^a</i>	Erythroid differentiation regulator 1	1.38	1.36	1.40	1.26
<i>Sgk^a</i>	Serum/glucocorticoid regulated kinase	1.12	1.82	1.19	1.42
<i>C10orf10^a</i>	Chromosome 10 open reading frame 107	1.29	1.20	1.12	1.23
<i>Ly6c</i>	Lymphocyte antigen 6 complex, locus C	1.13	1.46	1.12	1.19
<i>Sucla2</i>	Succinate-CoA ligase, ADP-forming, beta subunit	1.13	1.38	1.14	1.10
<i>b) Genes showing >10% decreases in each model</i>					
<i>Qdpr^a</i>	Quinoid dihydropteridine reductase	0.72	0.88	0.68	0.87
<i>2310075G12Rik</i>	Translation of histone mRNAs	0.82	0.75	0.82	0.90
<i>c) Increases and decreases >20% in each model</i>					
<i>Ccl21a</i>	Chemokine (C–C motif) ligand 21a (leucine)	0.54	0.69	3.90	0.72
<i>Ccl21b</i>	Chemokine (C–C motif) ligand 21b (serine)	0.51	0.75	3.31	0.73
<i>Ccl21c</i>	Chemokine (C–C motif) ligand 21c (leucine)	0.47	0.80	4.09	0.71
<i>Egr2^a</i>	Early growth response 2 (Egr2)	0.51	2.70	1.27	1.52
<i>Egr4^a</i>	Early growth response 4 (Egr4)	0.71	1.94	1.26	1.51
<i>Arc^a</i>	Activity regulated cytoskeletal-associated protein	0.58	1.46	1.21	2.23
<i>Wars2</i>	Tryptophanyl tRNA synthetase 2 (mitochondrial)	1.25	0.74	1.91	1.24
<i>Krt1–12</i>	Keratin complex 1, acidic, gene 12	0.74	1.21	1.25	0.24

^a Significant differential expression reported in published array studies.

involved in responses to cellular stress. Increased expression of this gene was also reported by [Kajiyama et al. \(2010\)](#) in a mouse model of prednisone-induced depression. Another pro-survival gene *Mpk-1* (mitogen-activated protein kinase phosphatase-1; *Dusp1*) which is regulated by glucocorticoids ([Hunsberger et al., 2009](#)) was also differentially up-regulated in 3 models by 19–

65%. The up-regulated *Ly6c* (lymphocyte antigen 6 complex, locus C) is an antigen on microglial precursor cells. The differentially increased expression of these genes in all models suggests that cellular stress, remodelling or degeneration, which involve such genes, are shared features in the four GM models we selected in our studies.

Table 3 Genes differentially regulated across models significantly enriched in the Synapse GO category. Genes showing greater than 10% deviation in 3 models underlined, in all 4 models in bold. Amino-acid receptors shown for interest. Numbers are fold changes. The complete list can be found in Supplementary material.

Post-synaptic elements		CNR1	VGLUT1	HTT	GRI	Av Dv
<i>Arc</i>	Activity regulated cytoskeletal-associated protein	0.58	1.46	1.21	2.23	0.58
<i>Lin7b</i>	Lin-7 homolog b (c. Elegans)	1.23	1.26	1.16	0.88	0.19
<i>Klh17</i>	Kelch-like 17 (drosophila)	0.69	0.70	0.98	1.19	0.20
<i>Lin7a</i>	Lin-7 homolog a (c. Elegans)	1.14	1.44	0.90	0.97	0.18
<i>Pick1</i>	Protein interacting with c kinase 1	1.01	1.27	1.14	0.90	0.13
<i>Pre synaptic elements</i>						
<i>Sv2a</i>	Synaptic vesicle glycoprotein 2 a	1.26	0.71	1.36	1.14	0.26
<i>Amino-acid receptors</i>						
<i>Gabrg2</i>	Gamma-aminobutyric acid receptor a, subunit gamma 2	1.07	1.42	1.20	0.94	0.19
<i>Gabra3</i>	Gamma-aminobutyric acid receptor a, subunit alpha 3	1.00	1.52	0.96	0.96	0.15
<i>Gabbra1</i>	Gamma-aminobutyric acid receptor b, 1	0.94	1.49	0.98	1.00	0.14
<i>Grm1</i>	Glutamate receptor, metabotropic 1	1.01	0.54	0.99	1.14	0.15
<i>Gria1</i>	Glutamate receptor, ionotropic, ampa1 (alpha 1)	1.00	0.61	1.06	0.98	0.12
<i>Grik5</i>	Glutamate receptor, ionotropic, kainate 5 (gamma 2)	1.11	0.66	0.98	1.00	0.12
<i>Miscellaneous</i>						
<i>Nefm</i>	Neurofilament 3, medium	0.84	1.31	1.08	0.80	0.18
<i>Cbln4</i>	Cerebellin 4 precursor protein	0.63	1.12	1.00	1.12	0.15
<i>Ache</i>	Acetylcholinesterase	1.11	0.67	0.97	1.12	0.15

AvDv: average positive or negative deviation from fold change of 1.0.

5.2. Differentially down-regulated genes

Quinoid dihydropteridine reductase (*Qdpr*) showed differentially reduced expression in all 4 models, with greater effects seen in the CNR1 and HTT models. Decreased expression of this gene was reported by [Surget et al. \(2009\)](#) in the ultra-mild chronic stress model. The *Qdpr* enzyme recycles the co-factor tetrahydrobiopterin (BH4) after its use by phenylalanine, tyrosine and tryptophan hydroxylases, the rate-limiting steps in the synthesis of monoamine neurotransmitters. Whether the small changes in the 4 GM models are sufficient to affect monoamine synthesis is a matter of speculation but this seems unlikely since 5-HT synthesis is increased in the CNR1 model ([Aso et al., 2009](#)). There are some inconsistent reports of reduced circulating and CSF levels of BH4 in an older literature in depression ([Abou-Saleh et al., 1995](#)). BH4 is also a co-factor for all forms of nitric oxide synthase (NOS) and BH4 deficiency may result in the formation of reactive oxygen species ([Schmidt and Alp, 2007](#)). This could be a mechanism for oxidative stress in the CNS as inferred from the expression changes reported by [Hovatta et al. \(2005\)](#).

5.3. Differentially up and down-regulated genes and synapse genes

Three CCL21 [chemokine (C–C motif) ligand 21]-related chemokine genes were up-regulated more than 3-fold in the HTT model but, in contrast, appreciably reduced in expression in the other three models. Several other cytokine, interleukin and interferon genes showed differential expression across the models but these changes were not obviously more marked in the HTT model. Thus CCL21 signalling appears to be a specific process involved in the HTT model. Neuronal CCL21 is a microglia-activating chemokine, which is expressed by endangered neurons in the brain under stress conditions and can be either neuroprotective or damaging ([de Jong et al., 2008](#)). Therefore, the observed increases and the decreases in our four GM models may relate to the growing evidence that cytokine dysfunction can play a role in the pathogenesis of depression ([Dinan, 2009](#)).

Three genes associated with synaptic plasticity showed more than 20% changes in all models; early growth response 2 and 4 genes (*Egr2*, *Egr4*) and activity-related cytoskeletal-associated (*Arc*) protein encoding gene ([Table 2c](#)). Furthermore, a very similar pattern of responses with 16–20% changes was seen for *Egr1* in three of the four models. In each case expression of these genes was decreased in the CNR1 model and increased to a similar extent in the other 3. *Egr 2* was one of 9 genes up-regulated (confirmed by qPCR) in the frontal cortex of rats exposed to the chronic mild stress (CMS) paradigm causing loss of sucrose preference ([Orsetti et al., 2008](#)). *Egr 1–4* belong to a family of highly conserved and rapidly inducible genes coding for transcription factors that are implicated in long-term potentiation (LTP) and various forms of learning and memory-related behaviours motivated by addictive drugs or aversive stimuli ([Poirier et al., 2007](#)). *Arc* in contrast is not a transcription factor but an effector protein and is also necessary for LTP and implicated in various forms of learning ([Bramham, 2007](#); [Bramham et al., 2010](#)). Antidepressant drugs induce expres-

sion of *Arc* and *Erg* genes ([Hunsberger et al., 2007](#); [Pei et al., 2003](#); [Slattery et al., 2005](#)). However, a casual role in depressive behaviour has yet to be demonstrated. Indeed, in view of their response to antidepressants and their mediating role in synaptic plasticity, it is puzzling that in three of the GM models and the CMS model ([Orsetti et al., 2008](#)) these genes showed increased expression. The explanation may be provided by evidence that increased *Arc* expression is a resilience mechanism, in line with the report of [Kozlovsky et al. \(2008\)](#) that mice showing least behavioural disruption after exposure to cat odour showed the greatest increase in *Arc* expression compared to controls. If *Arc* and *Egr* act as resilience mechanisms, this would suggest that it is the CNR1 model in which impaired *Arc* and *Egr* effects could play a causal role in depression-related behaviours.

Arc and *Egr* immediate early genes are regulated by multiple intracellular kinases such as MAPK and CaMK, and de-phosphorylation by phosphatases such as *Dusp6* (dual specificity phosphatase 6). A number of these genes appear in the differentially regulated list including *Dusp6* which negatively regulates the activity of MAP kinases and showed changes of expression from 9 to 42% across the models. However, *Mapk* gene expression did not show general changes (Supplementary material). Intracellular kinases and phosphatases in turn mediate the effect of extracellular ligand-receptor interactions such as BDNF-TrkB and glutamate-NR/mGluR interaction. Few 'classic' ligands and receptors appeared in the differentially regulated list; changes in expression of GABA and glutamate receptor subunits were confined to the VGLUT1 model probably reflecting the disruption of presynaptic vesicular storage and release.

During synaptic activity, *Erg* and other transcription factors regulate the expression of genes such as *Arc*, whose mRNA is rapidly translated in the soma and dendrites. Key members of the cap-dependent mRNA translation machinery and direct targets of MAPK and mTOR signalling, including *eIF4E* (eukaryotic translation initiation factor 4E), *eIF4BP2*, ribosomal protein S6 kinase and ribosomal protein S6 were differentially regulated. In neurons, mTOR is up-regulated in response to growth factors and neurotransmitters (such as BDNF and glutamate) in an activity-dependent manner and modulates the synthesis of local proteins such as *Arc* and *CAMKII* during LTP and LTD and memory formation ([Costa-Mattioli et al., 2009](#); [Slipczuk et al., 2009](#)). The changes in expression of the main down-stream targets of mTOR and MAPK signalling, together with robust changes in *Arc* mRNA, suggest that local mRNA processing mechanisms at dendritic spines may be disturbed in the GM models. Indeed [Hajszan et al. \(Hajszan et al., 2009\)](#) reported remodelling of hippocampal dendritic spines that correlated with learned helplessness after stress and its reversal by antidepressants.

6. Conclusion

Our main finding is that genetically modified mouse models of depression shared common signs of oxidative/cellular stress and synaptic remodelling in the consistent but variable up-regulation across the models of relevant genes and the down-regulation seen in others. In line with these observations the differentially expressed genes were enriched in gene ontology (GO) categories that are related to metabolic,

both anabolic and catabolic processes and energy demand together with enrichment in the synapse GO category. The synaptic genes were not related to traditionally suspected neurotransmitter systems but rather to genes that are relevant for post-synaptic signal transduction and neuronal connectivity.

In conclusion we describe shared changes in gene expression across four GM mouse strains that show a depression-like behavioural phenotype. Several changes replicate previous observations in the animal literature and some in human post-mortem brain. This replication approach offers a method of validating array findings in addition to real-time PCR which has its own uncertainties (Allison et al., 2006) and to in-situ hybridisation.

Studies of the effects of antidepressants on the shared changes are in progress to identify which are critical in mediating the depression-like behavioural phenotype and which are adaptive changes. The changes may also identify new targets for drug-treatment as suggested by the recent finding that the antidepressant-like effects of ketamine but not traditional antidepressants, depend on engaging molecular cascades underlying synapse formation including some of the genes identified in this study (Li et al., 2010).

Supplementary materials related to this article can be found online at [doi:10.1016/j.euroneuro.2010.09.011](https://doi.org/10.1016/j.euroneuro.2010.09.011).

Role of the funding source

This study was supported by the Sixth Framework Program of the EU, NewMood, LSHM-CT-2004-503474. The funding source had no further role in study design; in the collection, analysis and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Fabre, del Rio, Hamon, Lanfumey, Lesch, Maldonado, Serra, Sharp, Tordera, and Deakin participated in the design of the study. Hoyle, Juhasz, Chase, Toro and Deakin drafted the manuscript. Hoyle performed the statistical analysis. Deakin, Juhasz and Toro searched gene databases and interpreted the results. Aso, del Rio, Fabre, Hamon, Lanfumey, Lesch, Maldonado, Serra, Sharp, Tordera contributed substantially to the data acquisition and data handling and revised the manuscript. All authors read and approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgement

We wish to acknowledge the help of Dr I Deakin in using the Gene Ontology David analysis.

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