

Molecular Signatures of Mood Stabilisers Highlight the Role of the Transcription Factor REST/NRSF

Alix Warburton^a, Abigail L. Savage^a, Paul Myers^a, David Peeney^b, Vivien J. Bubb^a, John P. Quinn^{a,*}.

^a Department of Molecular and Clinical Pharmacology, The University of Liverpool, Liverpool, UK

^b Department of Physiology, The University of Liverpool, Liverpool, UK

* Corresponding author: Department of Molecular and Clinical Pharmacology, The University of Liverpool, Liverpool, UK, L69 3BX. Tel: +44 151 794 5498. Fax: +44 151 794 5517. E-mail:

jquinn@liverpool.ac.uk

Abstract

Background: The purpose of this study was to address the affects of mood modifying drugs on the transcriptome, in a tissue culture model, using qPCR arrays as a cost effective approach to identifying regulatory networks and pathways that might coordinate the cell response to a specific drug.

Methods: We addressed the gene expression profile of 90 plus genes associated with human mood disorders using the StellARray™ qPCR gene expression system in the human derived SH-SY5Y neuroblastoma cell line. *Results:* Global Pattern Recognition (GPR) analysis identified a total of 9 genes (DRD3*, FOS[†], JUN*, GAD1*[†], NRG1*, PAFAH1B3*, PER3*, RELN* and RGS4*) to be significantly regulated in response to cellular challenge with the mood stabilisers sodium valproate (*) and lithium ([†]). Modulation of FOS and JUN highlight the importance of the activator protein 1 (AP-1) transcription factor pathway in the cell response. Enrichment analysis of transcriptional networks relating to this gene set also identified the transcription factor neuron restrictive silencing factor (NRSF) and the oestrogen receptor as an important regulatory mechanism. *Limitations:* Cell line models offer a window of what might happen *in vivo* but have the benefit of being human derived and homogenous with regard to cell type. *Conclusions:* This data highlights transcription factor pathways, acting synergistically or separately, in the modulation of specific neuronal gene networks in response to mood stabilising drugs. This model can be utilised in the comparison of the action of multiple drug regimes or for initial screening purposes to inform optimal drug design.

Key words: Global Pattern Recognition; mood disorders; mood-modifying drugs; neuronal signalling; NRSF; pathway analysis

¹ *Abbreviations:* AP-1, activator protein 1; ENCODE, Encyclopaedia of DNA Elements; ERK, extracellular-signal-regulated kinase; GPR, Global Pattern Recognition; GxE, Gene x Environment; NRSF, neuron restrictive silencing factor; REST, repressor element-1 silencing transcription factor

1. Introduction

Mental health is in part dependent upon transcriptional responses to cues which can be environmental, chemical, physiological and psychological; this is termed the Gene x Environment (GxE) component. These changes not only affect our health in the short term, but can have medium to long term impact via epigenetic modulation of gene expression, altering our response to environmental challenges. Genetic polymorphism can modulate the GxE response and offer insight into the mechanisms underpinning such pathways (Quinn et al., 2013). Earlier genetic studies targeted association of one genetic variant to a specific disorder; this had limited success and focused predominantly on candidate genes such as those in the monoaminergic pathways. These correlations are now being readdressed by analysing multiple variants in such pathways or by stratification of the cohorts based on environmental factors. Our recent work on a promoter polymorphism of the monoamine oxidase A gene and maternal parameters affecting infant behaviour is an example of the latter (Hill et al., 2013). It is difficult to address the signal cascade in response to specific challenges *in vivo* due to the heterogeneity of cells involved in processing the environmental signals mediating a cellular response. However, *in vitro* cell line models offer an opportunity to address in fine detail the signal pathways modulated in response to a specific challenge. In this study we analysed the response to distinct drugs in the human neuroblastoma cell line SH-SY5Y targeting a commercially available compilation of mood disorder genes to address whether they leave a molecular signature of transcriptional change to the challenge. The drugs chosen for comparison included two psychostimulant challenges, amphetamine and cocaine, and two mood stabilisers, sodium valproate and lithium. All of these drugs have been shown previously to modulate signal pathways in SH-SY5Y cells at the transcriptional and/or post-transcriptional level (Asghari et al., 1998; Di Daniel et al., 2005; Kantor et al., 2002; Lew, 1992; Pan et al., 2005; Warburton et al., 2014). Our analysis identified similarities and differences in the networks modified by the drug challenge which suggested an overlap in the pathways of the mood stabilisers. These changes reflect one window for the spectrum of changes that could occur *in vivo*, but nonetheless outline the potential for a concerted cellular response to drug exposure.

2. Materials and methods

2.1. Cell culture and drug treatment

Human derived SH-SY5Y neuroblastoma cells (American Type Culture Collection) were maintained in Earle's modified Eagle's medium (EMEM) (Sigma) and HAM's F12 (Sigma) at a ratio of 1:1, supplemented with 10% foetal calf serum (FCS) (Sigma), 1% 200mM L-glutamine, 1% 100mM sodium pyruvate and 100 U/ml penicillin /100 ug/ml streptomycin at 37°C and 5% CO₂. Amphetamine, cocaine hydrochloride, lithium chloride and valproic acid sodium salt were purchased from Sigma and stock solutions made using sterile filtered dH₂O. Drug regimes were 1 hour treatment with either: vehicle control (sterile filtered dH₂O), 10µM amphetamine (Jones and Kauer, 1999; Shyu et al., 2004), 10µM cocaine (Warburton et al., 2014), 1mM lithium (Hing et al., 2012; Roberts et al., 2007) or 5mM sodium valproate (Pan et al., 2005; Phiel et al., 2001; Zhang et al., 2003). For each drug treatment, n=4. Basal (untreated) cells were also included.

2.2. RNA extraction and quantitative polymerase chain reaction (qPCR) analysis

Total RNA was extracted using Trizol reagent (Invitrogen) and the resulting RNA pellets resuspended in RNase-free water. 500ng RNA was reverse transcribed into cDNA using the GoScript™ RT system (Promega). qPCR analysis was performed on an iQ5 real-time PCR system (Bio-Rad) using 1µl of cDNA per reaction and GoTaq® qPCR Master Mix (Promega) with the addition of Fluorescence Calibration Dye (Bio-Rad) at a final concentration of 10nM. Changes in gene expression were analysed on the Lonza Web site (<http://array.lonza.com/gpr>), using the Global Pattern Recognition™ (GPR) analysis software designed by Bar Harbor Biotechnology (<https://www.bhbio.com/BHB/dw/home.html>). This algorithm internally normalised the real-time qPCR data set of each gene with respect to all genes within the experiment and generated a list of genes that are ranked on the basis of the difference between the test and control expression levels and the consistency of the data between the biological replicates. This proprietary software calculated both the fold-change data and the respective p-values. The results are displayed as change with respect to the genes that showed minimal changes, which were defined on Ct values obtained using the Global Pattern Recognition analysis software (Akilesh et al., 2003).

A list of genes on the mood array is given in Table 1.

2.3. Bioinformatic analysis

Gene expression data generated from GPR analysis was uploaded into the online biological pathway analysis software MetaCore™, version 6.15 build 62452. Functional enrichment of the experimental dataset was performed using: 1) the Pathway Map analysis tool to identify significantly associated pathways based on p-value and GPR Fold-change and 2) Build Network for Your Experimental Data feature using the Transcription Factor Targets Modelling algorithm with default settings under Analyse Networks (Transcription Factors) to generate sub-networks based on the presence of transcription factors and/or receptor targets within the original input file. Such genes/proteins uploaded from experimental datasets and from which pathways were built upon were termed 'seed nodes'.

In silico analysis of NRSF binding sites over the significantly altered genes across the different treatment conditions from the qPCR array data were identified using Transcription Factor ChIP-seq from ENCODE (Encyclopaedia of DNA Elements), version 4, available on the UCSC Genome Browser (<http://genome.ucsc.edu/index.html>). Upstream and downstream flank sequences (10 Kb) were included and the position of NRSF binding sites calculated. For genes with multiple transcripts, the locus for the largest isoform was used. The full list of NRSF binding sites are detailed in Table 3.

3. Results

3.1. Gene expression profiling of human SH-SY5Y cells in response to mood-modifying drugs using Global Pattern Recognition analysis

To investigate the effects of mood modifying drugs on the expression of a panel of genes associated with mood disorders (Human Mood Disorder 96 StellARray™), SH-SY5Y neuroblastoma cells after treatment for 1 hour under one of the specified conditions were analysed using the proprietary Global Pattern Recognition (GPR) algorithm which compares the change in expression of a gene normalised to the expression of every other gene in the array (Akilesh et al., 2003). This software calculates both the fold-change data and the respective p-values with respect to genes that showed minimal changes. We and others have recently demonstrated that drugs used in the treatment of mood disorders can differentially affect the expression stability of traditionally used housekeeping genes, impacting upon their usefulness as normalising factors (D'Souza et al., 2013; Powell et al., 2013; Sugden et al., 2010). Unfortunately, these large changes in gene expression may mask small but biologically important changes in gene expression, such as master regulator genes (e.g., transcription factors). The data in Table 2 therefore represents a more appropriate display of the genes most changed within the experiment by comparing all genes against themselves. As the array contains validated mood genes we addressed the top 10 genes which significantly changed in response to each drug to define pathways and networks within the larger gene list.

Following treatment with the mood stabiliser sodium valproate, 8 genes were significantly ($p < 0.05$) up- or down-regulated compared to the vehicle control; 2 up-regulated (JUN and PAFAH1B3) and 6 down-regulated (DRD3, GAD1, NRG1, PER3, RELN and RGS4). When compared to the results obtained after treatment with another common mood stabiliser, lithium, similarities in the gene expression profile with respect to the top 10 altered genes was observed; namely down-regulation of GAD1, NRG1, PER3, RELN and RGS4, but, only GAD1 reached statistical significance at this time point for lithium treatment. In addition, FOS was significantly down-regulated in response to lithium. Treatment with the two psychomotor stimulants cocaine and amphetamine demonstrated no statistically significant changes in gene expression following 1 hour treatment. Furthermore the genes with the lowest p-values were distinct between the psychostimulants apart from MOBP (Table 2)

demonstrating that these drugs might be preferentially targeting distinct pathways for their action. However due to the low p-values obtained under these experimental conditions we did not pursue their analysis further.

3.2. Network analysis of genes significantly modulated in response to mood stabilisers

To further explore potential gene networks important in the response to drug challenge, we analysed only the genes whose expression was most affected by lithium and sodium valproate using the Analyse Networks (Transcription Factors) algorithm from MetaCore™. This generates sub-networks through relative enrichment of the uploaded dataset based on the presence of transcription factors and/or receptor targets within the original input file. The gene set used was composed of GAD1, NRG1, PER3, RELN, RGS4, PAFAH1B3, DRD3, FOS and JUN, the first five of which were observed for both lithium and sodium valproate and the remaining were those significantly modified in response to either exposure.

A network containing NRSF, ErbB2 and ErbB3 as seed nodes was the highest ranked using this approach, and was defined as genes/proteins uploaded from experimental datasets or genes/proteins directly linked to uploaded gene lists from which networks are built (Figure 1). It included 7 of our 9 input genes (DRD3, FOS, GAD1, JUN, NRG1, PAFAH1B3 and RELN) and had a p-value of 5.24×10^{-29} based on hypergeometric distribution which calculated the probability of a particular pathway map arising by chance given the number of genes across all gene pathways, within a particular pathway or sub-network and within the present experimental dataset. The transcription factors identified as being important regulators of this network were c-Fos and c-Jun (collectively AP-1), c-Myc, ESR1, NRSF, PR, RAR-alpha and SP3.

As our gene expression data showed that 7/9 of the significantly modulated genes were down-regulated (Table 2) and NRSF which predominantly functions as a transcriptional repressor was identified as an important regulator of our gene set, we addressed predicted NRSF binding sites using ENCODE data from the Transcription Factor ChIP-seq track (2011; Rosenbloom et al., 2013) on the UCSC Genome Browser. This identified NRSF binding at the promoter regions (within 5 Kb of the transcriptional start site) of DRD3 (transcript variant a, e and g), FOS, GAD1, JUN, NRG1 (transcript

variant HRG-gamma1/2/3, HRG-beta1/d-, 2- and 3b, ndf43/b/c, HRG-alpha and SMDF), PAFAH1B3 and RGS4 (transcript variant 2/3) which, with the exception of JUN and PAFAH1B3, were all down-regulated in response to 1 hour treatment with sodium valproate (or lithium with respect to FOS).

To determine how these regulatory pathways were most relevant for mood disorders, we filtered our dataset using the MetaCore™ 'Filter by Disease' feature which traces all of the known associated interactions for a particular disease process. This assigned 46.15% of our network, not unexpectedly to disease processes relating to mood (Figure 2A). Furthermore, it identified NRSF and ERK1/2 signalling along the oestrogen receptor pathway as important regulators of processes relevant to mood disorders involving this subset of genes. In addition to disorders of the CNS, filtering of our dataset by disease showed there to be significant associations (96.15%) with breast, skin and gastrointestinal neoplasia; GAD1 being the only gene not to be involved in these cancer-related pathologies (Figure 2B). To further assess which signalling pathways may be operating in response to challenge with these mood stabilisers, we also filtered our experimental network for Drug Responses under the Gene Ontology (GO) Processes filter. This identified the fibroblast growth factor, ERBB and neurotrophin TRK receptor signalling pathways as important cellular responses, with the dopamine D3 receptor, EGFR, ErbB2, ErbB3 and c-Src highlighted as therapeutic targets (Figure 2C).

4. Discussion

Understanding the mechanism of action for a drug to alter the cell phenotype, in addition to the initial cellular targets recognised by the drug, is important for both clinical application and pharmaceutical development. Transcriptome profiling allows for global scale interrogation of potential regulatory mechanisms involved in modulating cellular responses to a particular drug through the use of pathway analysis tools. The aim of this study was to address the effects of mood modifying drugs on the expression profile of a commercially available panel of genes associated with mood disorders by network analysis to compare and contrast their mode of action.

We used two mood stabilisers (lithium and sodium valproate) and two mood stimulants (cocaine and amphetamine). Only the mood stabilisers reached statistical significance and interestingly they shared 5 genes in their top 9 most modified genes, Table 2; we therefore focused on this set of genes for further analysis. Valproate significantly modified 8 genes, lithium only two, GAD1 and FOS, with GAD1 being significantly down-regulated for both drugs. GAD1 encodes one of several forms of glutamic acid decarboxylase which is a key enzyme for the synthesis of the inhibitory neurotransmitter GABA. GAD1 is implicated from both genetic and functional analysis as a modulator of mood (Domschke et al., 2013; Hettema et al., 2006; Karolewicz et al., 2010; Lundorf et al., 2005; Thompson et al., 2009; Weber et al., 2012). FOS and JUN proteins constitute the AP-1 transcription factor complex which was a target for modulation. These factors represent a family of proteins that heterodimerise to regulate the AP-1 DNA site (Quinn, 1991; Quinn et al., 1989a; Quinn et al., 1989b; Takimoto et al., 1989). Lithium and sodium valproate have both been demonstrated to modulate the AP-1 complex (Chen et al., 2008; Ozaki and Chuang, 2002). The genes shared in common by the mood stabilisers sodium valproate and lithium were GAD1, NRG1, PER3, RELN and RGS4. The remainder, DRD3, JUN and PAFAH1B3 were specific for sodium valproate. Although some of these genes were modified with cocaine and amphetamine, the statistical significance was low, certainly lower than all the genes in the 9 most differentially expressed genes in Table 2. We have previously used cocaine and amphetamine in SH-SY5Y and found that we can observe significant changes in genes involved in mental health. For example, recently in the approximate same passage number of cells as used in this experiment, we have demonstrated that cocaine altered the expression of the

schizophrenia candidate gene MIR137 (Warburton et al., 2014). However under the current experimental conditions this gene set targeting mood disorders is not responding as robustly to cocaine and amphetamine as lithium and sodium valproate. We therefore attempted to determine whether the significant mood stabiliser gene set defined a specific pathway or network of genes to explain their concerted response to drug exposure.

Pathway analysis using both the Analyse Networks (Transcription Factors) and Filter by Disease algorithms available on the online pathway analysis software MetaCore™ identified the transcription factor NRSF, also termed REST (repressor element-1 silencing transcription factor), to be strongly associated with the pathways supporting these networks of genes. NRSF has a direct association with DRD3, GAD1 and RELN genes based on the network analysis, Figure 1. Bioinformatic analysis of predicted NRSF binding sites using ENCODE (Encyclopaedia of DNA Elements) data from the Transcription Factor ChIP-seq track (2011; Rosenbloom et al., 2013) on the UCSC Genome Browser, identified NRSF binding at the promoter regions (within 5 Kb of the transcriptional start site) of the FOS, NRG1 and RGS4 genes, Table 3. This ENCODE analysis also demonstrated NRSF binding sites in similar genomic locations on DRD3, GAD1, JUN, PAFAH1B3 and RELN. Aberrant signalling of NRSF and its target genes has been shown to be involved in the pathophysiology of several CNS disorders including schizophrenia (Loe-Mie et al., 2010), major depressive disorder (Otsuki et al., 2010) and alcoholism and depression (Ukai et al., 2009), with genetic variants influencing age-related cognitive function (Miyajima et al., 2008). More recently it has been highlighted as a major player in Alzheimer's disease (Lu et al., 2014). NRSF has the properties to modulate epigenetic factors in its target genes due to its association with a plethora of co-activators, such as members of the SWI/SNF family, which can modify histones by post-translational modifications (Loe-Mie et al., 2010). These epigenetic modifications could result in medium to long term changes in gene expression that underlie drug exposure in addition to the immediate modulation of the transcriptome. Our data suggest that lithium and sodium valproate, with different initial cellular targets, may modulate related signalling pathways leading to overlapping cellular responses mediated in part by the NRSF pathway. It should be noted that we performed this experiment at 1 hour post exposure to capture an early response of the cell to the drug. As in any stimulus induction modification of gene expression many of these changes will be transient, especially in the short term for transcription factors such as AP-1 and

NRSF. This is in keeping with the transient response of AP-1 and NRSF in stimulus inducible gene expression models we have previously observed at 1 hour post exposure (Gillies et al., 2009; Howard et al., 2008; Quinn, 1991; Spencer et al., 2006). A more extensive timescale would perhaps have demonstrated a different or related set of genes, nevertheless, our strategy allowed the observation of the differential gene set acting as a signature for the mood stabilisers and allows for future optimisation.

Filtering our dataset by disease also identified ERK1/2 signalling along with the oestrogen receptor pathway as a potentially important regulatory network for this gene set (Figure 2). Oestrogen receptor signalling has been well documented in the modulation of behaviours relating to aggression (Nomura et al., 2002), anxiety and depression (Furuta et al., 2013). The action of sex hormones may in part explain why in conditions such as panic disorder these phenotypes are more prevalent among females. Our data would be consistent with GAD1 SNP variation being tentatively associated for the higher susceptibility of females to panic disorder (Weber et al., 2012) via modulation by oestrogen. This oestrogen pathway could overlap with other transcription factor pathways identified in our analysis, for example synergistic action of the oestrogen and AP-1 pathways on gene expression (Fujimoto and Kitamura, 2004). The extended networks identified in this study (AP-1, oestrogen and NRSF) may also work synergistically, for example NRSF activity is important for E2 stimulation of the cell cycle (Bronson et al., 2010) and oestrogen receptor B is enriched at NRSF binding sites (Le et al., 2013). Such interactions between these three pathways can be further modified by the glucocorticoid receptor, so linking these pathways to a major driver of mood (Abramovitz et al., 2008; Karmakar et al., 2013). Glucocorticoid sensitivity is strongly associated with several mood related disorders (Spijker and van Rossum, 2012) and anti-glucocorticoid drugs have been used in the treatment of such conditions (Gallagher et al., 2008; Wolkowitz and Reus, 1999; Wolkowitz et al., 1999). Mood disorder susceptibility has also been linked to glucocorticoid signalling through its modulation of the stress response along the hypothalamic-pituitary–adrenal (HPA) axis (Lupien et al., 2009; Spijker and van Rossum, 2012).

Our data points to a cost effective and rapid assessment of expression changes in selected genes using GPR analysis, which can help delineate the pathways targeted by drugs to modify mood. In

particular, we have identified dopamine and glutamine pathways as being important; perhaps not unexpectedly as the gene set is enriched for known genes involved in mood disorders. Alteration in the regulation of these pathways would be expected to modulate mood and is reflected in the range of drugs currently used in targeting these pathways. However the modulation of the AP-1 pathway and the involvement of factors such as NRSF and ERK1/2 highlight a more general modulation of neurotransmitter pathways in response to mood modifying drugs. Our model can therefore be used to determine mechanisms associated with off target and long term affects of particular drugs and can be extrapolated to predict *in vivo* responses, utilised in the comparison of multiple drug regimes or used as an initial screening process to inform optimal drug design.

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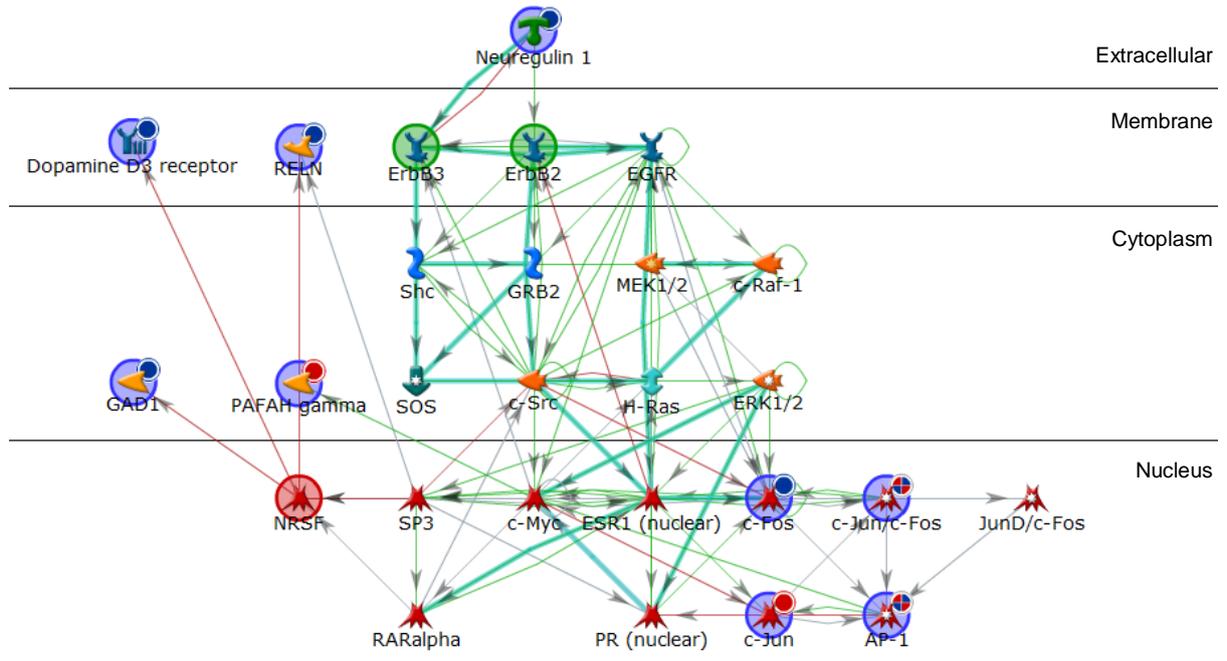
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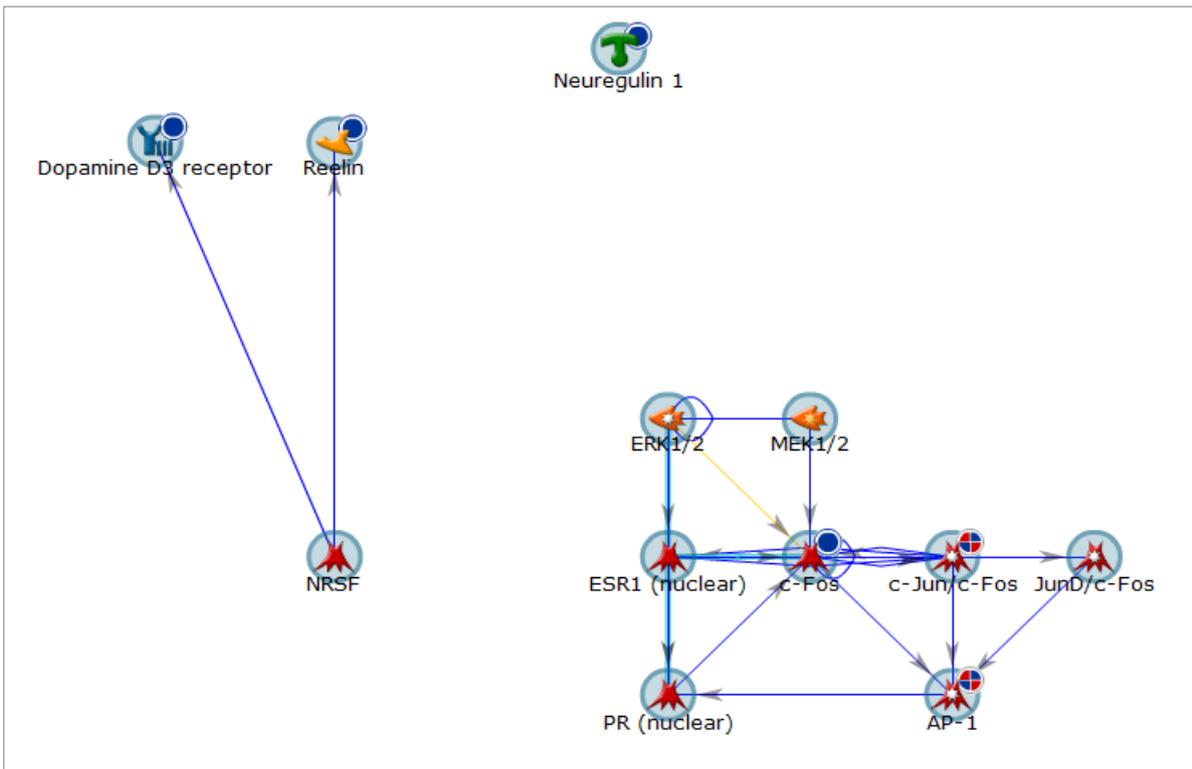
Figure 1. Network Analysis of genes significantly modulated in response to mood stabilisers. Genes shown to be significantly up or down regulated in human SH-SY5Y cells in response to 1 hour treatment with the mood stabilisers sodium valproate and lithium were uploaded into MetaCore™ for Network Analysis. The gene list was analysed under the Build Network feature using the Transcription Factor Targets Modelling algorithm. Seed nodes from which the network was built upon are encompassed by a large circle; blue circles represent genes from the experimental data, green circles represent molecules from which the pathway is expanded from and red circles represent molecules on which the pathway terminates. Genes uploaded from the experimental data are also marked with a smaller circle in their top right hand corner; red circles represent genes that were significantly up-regulated, whereas blue circles represent genes significantly down-regulated. Connecting arrows indicate interactions; green arrows represent activation, red arrows represent inhibition and blue arrows are unspecified. Overlaid cyan lines represent canonical pathways. Gene names/symbols within the network from top to bottom, left to right: Neuregulin 1, Dopamine D3 receptor, RELN, ErbB3, ErbB2, EGFR, Shc, GRB2, MEK1/2, c-Raf-1, GAD1 PAFAH gamma, SOS, c-Src, H-Ras, ERK1/2, NRSF, SP3, c-Myc, ESR1 (nuclear), c-Fos, c-Jun/c-Fos, JunD/c-Fos, RARalpha, PR (nuclear) c-Jun, and AP-1.

Figure 2. Network analysis filters for disease and gene ontology processes. The network generated in relation to genes significantly regulated in response to SH-SY5Y cell treatment with sodium valproate and lithium (**Figure 1**) was filtered to show the relevant disease pathways (A and B) and gene ontology processes (C). A-B, Disease processes relevant to mood disorders (A), represents 46.15% of the gene network; and breast, skin and gastrointestinal neoplasms (B), represents 96.15% of the gene network. C, Gene ontology processes relevant to drug response. Seed nodes from which the network was built upon are encompassed by a large blue circle. Genes uploaded from the experimental data are also marked with a smaller circle in their top right hand corner; red circles represent genes that were significantly up-regulated, whereas blue circles represent genes significantly down-regulated. Connecting blue arrows indicate direct interactions, yellow arrows indicate interactions that are in the base but do not form part of the network and overlaid cyan lines represent canonical pathways. Gene names/symbols within network A, from top to bottom, left to right: Neuregulin 1, Dopamine D3 receptor, Reelin, ERK1/2, MEK1/2, NRSF, ESR1 (nuclear), c-Fos, c-Jun/c-Fos, JunD/c-Fos, PR (nuclear) and AP-1; B, from top to bottom, left to right: Neuregulin 1, Dopamine D3 receptor, Reelin, ErbB3, ErbB2, EGFR, SOS, Shc, GRB2, c-Raf-1, PAFAH gamma, H-Ras, c-Src, ERK1/2, MEK1/2, NRSF, SP3, c-Myc, ESR1 (nuclear), c-Fos, c-Jun/c-Fos, JunD/c-Fos, RARalpha, PR (nuclear), c-Jun and AP-1; and C, from top to bottom, left to right: Dopamine D3 receptor, Reelin, ErbB3, ErbB2, EGFR, GAD1, c-Src, NRSF, c-Myc, c-Fos, c-Jun/c-Fos, JunD/c-Fos, c-Jun and AP-1.

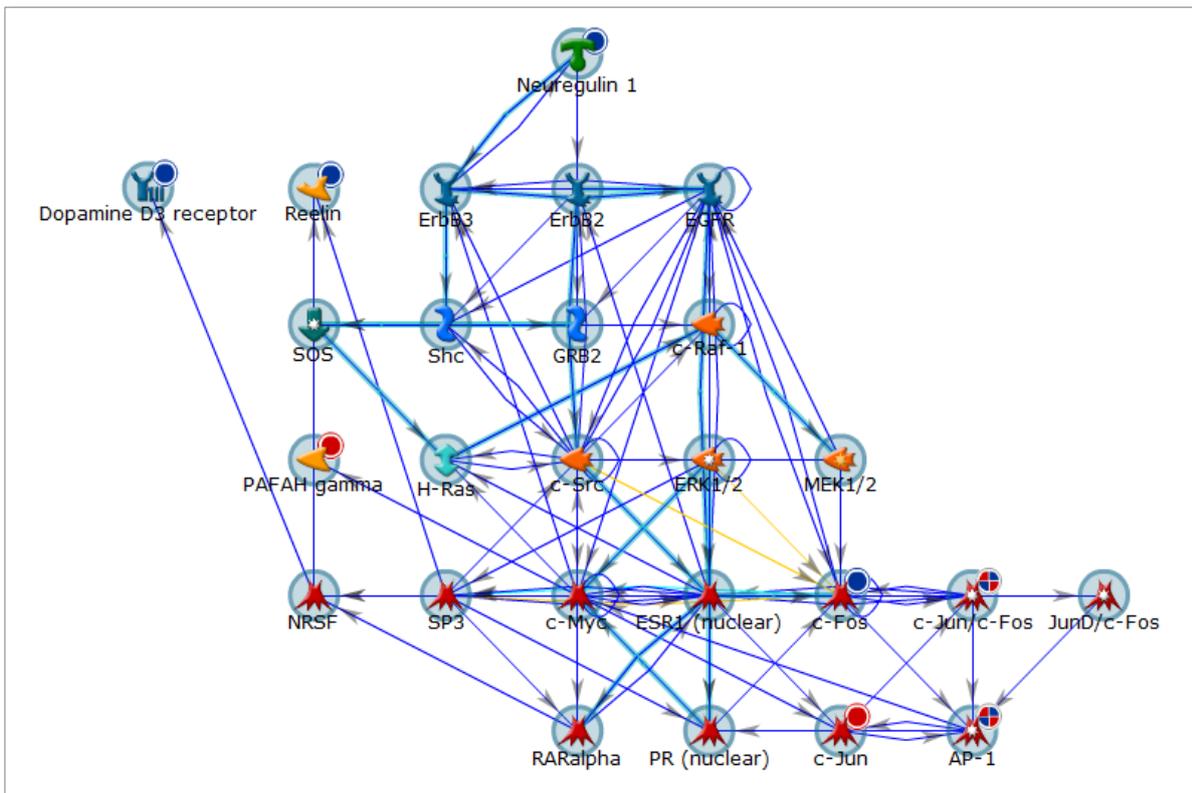
Figure(s)



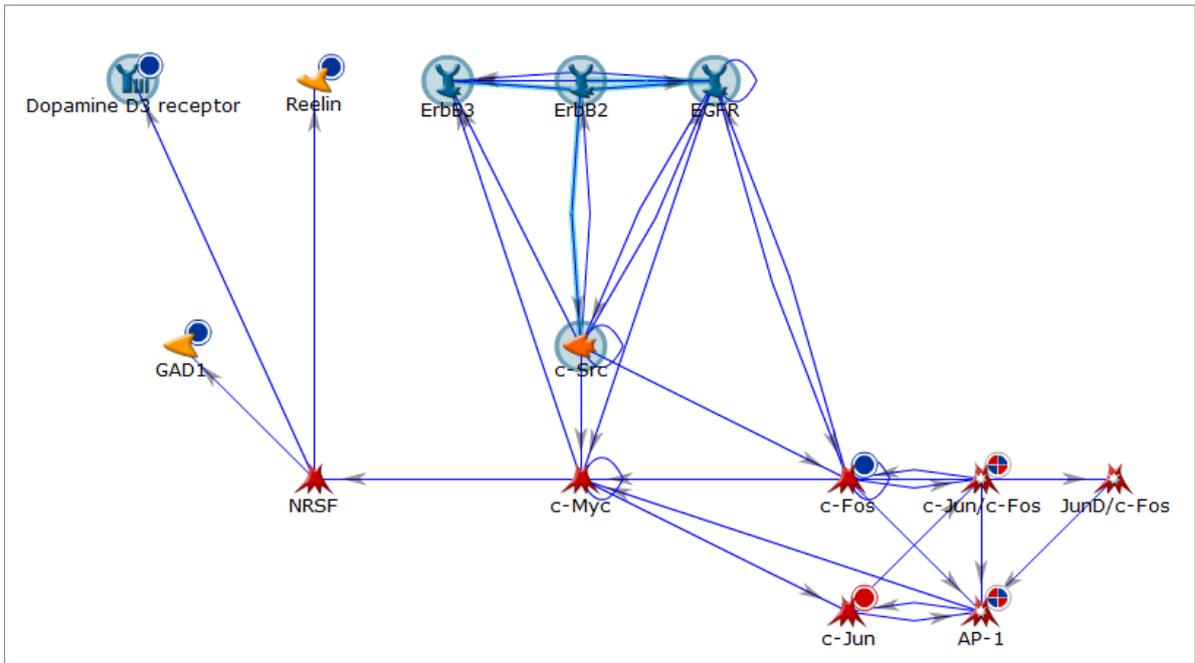
A



B



C



NETWORK OBJECTS

GENERIC CLASSES

-  Receptor ligand
-  Transcription factor
-  Generic binding protein

ENZYMES

-  Generic enzyme

KINASE

-  Generic kinase
-  Protein kinase

PROTEASE

-  Generic protease

GTPASE

-  RAS - superfamily

RECEPTORS

-  GPCR
-  Receptors with kinase activity

G PROTEIN ADAPTOR/REGULATORS

-  Regulators (GDI, GAP, GEF, etc.)

GROUPS OF OBJECTS

-  A complex or a group
Proteins physically connected into a complex or related as a family
-  Logical association
Proteins linked by logical relations or physical interactions

Table 1. Gene name and description for the Human Mood Disorder 96-well qPCR StellarRay™

Gene Name	Entrez Gene	Description
ACE	1636	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1
ADCYAP1	116	Adenylate cyclase activating polypeptide 1 (pituitary)
ADRBK2	157	Adrenergic, beta, receptor kinase 2
ARNTL	406	Aryl hydrocarbon receptor nuclear translocator-like
ATP2A2	488	ATPase, Ca ⁺⁺ transporting, cardiac muscle, slow twitch 2
BCR	613	Breakpoint cluster region
BDNF	627	Brain-derived neurotrophic factor
CASP8	841	Caspase 8, apoptosis-related cysteine peptidase
CCND2	894	Cyclin D2
CHRNA7	1139	Cholinergic receptor, nicotinic, alpha 7
CIT	11113	Citron rho-interacting serine/threonine kinase
CLOCK	9575	Clock circadian regulator
COMT	1312	Catechol-O-methyltransferase
CREB1	1385	CAMP responsive element binding protein 1
CRH	1392	Corticotropin releasing hormone
CRHBP	1393	Corticotropin releasing hormone binding protein
DAO	1610	D-amino-acid oxidase
DISC1	27185	Disrupted in schizophrenia 1
DLX1	1745	Distal-less homeobox 1
DRD1	1812	Dopamine receptor D1
DRD3	1814	Dopamine receptor D3
DRD4	1815	Dopamine receptor D4
DTNBP1	84062	Dystrobrevin binding protein 1
ERBB3	2065	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3
FAT1	2195	FAT atypical cadherin 1
FKBP5	2289	FK506 binding protein 5
FOS	2353	FBJ murine osteosarcoma viral oncogene homolog
GABRA5	2558	Gamma-aminobutyric acid (GABA) A receptor, alpha 5
GAD1	2571	Glutamate decarboxylase 1 (brain, 67kDa)
GCH1	2643	GTP cyclohydrolase 1
GPR50	9248	G protein-coupled receptor 50
GRIK3	2899	Glutamate receptor, ionotropic, kainate 3
GRIK4	2900	Glutamate receptor, ionotropic, kainate 4
GRIN2B	2904	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B
GRM3	2913	Glutamate receptor, metabotropic 3
GRM4	2914	Glutamate receptor, metabotropic 4
GSK3B	2932	Glycogen synthase kinase 3 beta
Hs18s	-	Human 18S ribosomal RNA
HS Genomic	-	Human genomic DNA control
HSP90B1	7184	Heat shock protein 90kDa beta (Grp94), member 1
HSPA5	3309	Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
HTR1B	3351	5-hydroxytryptamine (serotonin) receptor 1B
HTR2A	3356	5-hydroxytryptamine (serotonin) receptor 2A
IL1RN	3557	Interleukin 1 receptor antagonist
IMPA1	3612	Inositol(myo)-1(or 4)-monophosphatase 1
IMPA2	3613	Inositol(myo)-1(or 4)-monophosphatase 2
INPP1	3628	Inositol polyphosphate-1-phosphatase
ISYNA1	51477	Myo-inositol 1-phosphate synthase A1
JUN	3725	Jun oncogene
KCNN3	3782	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3
MAG	27307	Malignancy-associated gene

MAL	4118	Mal, T-cell differentiation protein
MAOA	4128	Monoamine oxidase A
MLC1	23209	Megalencephalic leukoencephalopathy with subcortical cysts 1
MOBP	4336	Myelin-associated oligodendrocyte basic protein
MOG	4340	Myelin oligodendrocyte glycoprotein
MTHFR	4524	5,10-methylenetetrahydrofolate reductase (NADPH)
NAPG	8774	N-ethylmaleimide-sensitive factor attachment protein, gamma
NCAM1	4684	Neural cell adhesion molecule 1
ND4	4538	Mitochondrially encoded NADH dehydrogenase 4
NDUFV1	4723	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa
NDUFV2	4729	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa
NOS1AP	9722	Nitric oxide synthase 1 (neuronal) adaptor protein
NR1D1	9572	Nuclear receptor subfamily 1, group D, member 1
NR3C1	2908	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
NRG1	3084	Neuregulin 1
NTRK2	4915	Neurotrophic tyrosine kinase, receptor, type 2
OLIG2	10215	Oligodendrocyte lineage transcription factor 2
P2RX7	5027	Purinergic receptor P2X, ligand-gated ion channel, 7
PAFAH1B1	5048	Platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa
PAFAH1B3	5050	Platelet-activating factor acetylhydrolase, isoform Ib, gamma subunit 29kDa
PCNT	5116	Pericentrin
PDLIM5	10611	PDZ and LIM domain 5
PER3	8863	Period circadian clock 3
PIP4K2A	5305	Phosphatidylinositol-5-phosphate 4-kinase, type II, alpha
PLA2G1B	5319	Phospholipase A2, group IB (pancreas)
PLA2G4A	5321	Phospholipase A2, group IVA (cytosolic, calcium-dependent)
PLCG1	5335	Phospholipase C, gamma 1
PLP1	5354	Proteolipid protein 1
POLG	5428	Polymerase (DNA directed), gamma
PTGS2	5743	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
RELN	5649	Reelin
RFX4	5992	Regulatory factor X, 4 (influences HLA class II expression)
RGS4	5999	Regulator of G-protein signaling 4
SLC12A6	9990	Solute carrier family 12 (potassium/chloride transporters), member 6
SLC6A2	6530	Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2
SLC6A3	6531	Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3
SLC6A4	6532	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
SULT1A1	6817	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1
SYNGR1	9145	Synaptogyrin 1
TAAR6	319100	Trace amine associated receptor 6
TF	7018	Transferrin
TIMELESS	8914	Timeless circadian clock
TPH1	7166	Tryptophan hydroxylase 1 (tryptophan 5-monoxygenase)
TPH2	121278	Tryptophan hydroxylase 2
XBP1	7494	X-box binding protein 1

Table 2. Gene expression profiling of SH-SY5Y cells following exposure to drugs affecting mood.

Lithium				Sodium Valproate			
Gene	Description	<i>p</i>	Fold change	Gene	Description	<i>p</i>	Fold change
FOS	FBJ murine osteosarcoma viral oncogene homolog	0.012	-2.57	DRD3	Dopamine receptor D3	0.001	-7.98
GAD1	Glutamate decarboxylase 1	0.023	-3.48	RGS4	Regulator of G-protein signaling 4	0.007	-2.08
RGS4	Regulator of G-protein signaling 4	0.063	-1.51	JUN	Jun oncogene	0.008	2.49
PER3	Period circadian clock 3	0.067	-1.38	RELN	Reelin	0.012	-1.78
NRG1	Neuregulin 1	0.068	-1.43	PER3	Period circadian clock 3	0.026	-1.48
NR1D1	Nuclear receptor subfamily 1, group D, member 1	0.069	-1.48	PAFAH1B3	Platelet-activating factor acetylhydrolase, isoform Ib, gamma subunit 29kDa	0.034	1.61
RELN	Reelin	0.078	-1.94	GAD1	Glutamate decarboxylase 1	0.035	-7.45
ACE	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	0.099	1.31	NRG1	Neuregulin 1	0.044	-1.34
Hs18s	Human 18S ribosomal RNA	0.105	1.64	MTHFR	Methylenetetrahydrofolate reductase (NADPH)	0.083	1.53
BDNF	Brain-derived neurotrophic factor	0.106	-1.39	RFX4	Regulatory factor X, 4 (influences HLA class II expression)	0.092	-1.49
Cocaine				Amphetamine			
Gene	Description	<i>p</i>	Fold change	Gene	Description	<i>p</i>	Fold change
SULT1A1	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	0.088	1.68	MOBP	Myelin-associated oligodendrocyte basic protein	0.080	2.08
DRD3	Dopamine receptor D3	0.110	-2.08	XBP1	X-box binding protein 1	0.093	1.34
FOS	FBJ murine osteosarcoma viral oncogene homolog	0.142	-1.45	NR1D1	Nuclear receptor subfamily 1, group D, member 1	0.109	-1.35
MOBP	Myelin-associated oligodendrocyte basic protein	0.161	1.85	MAG	Malignancy-associated gene	0.138	2.81
SLC6A2	Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	0.176	-1.28	PAFAH1B3	Platelet-activating factor acetylhydrolase, isoform Ib, gamma subunit 29kDa	0.141	1.33
GRIK3	Glutamate receptor, ionotropic, kainate 3	0.194	-1.67	FKBP5	FK506 binding protein 5	0.159	-1.34
TIMELESS	Timeless circadian clock	0.200	-1.20	RELN	Reelin	0.198	-1.30
NCAM1	Neural cell adhesion molecule 1	0.206	-1.20	BCR	Breakpoint cluster region	0.207	1.22
ND4	Mitochondrially encoded NADH dehydrogenase 4	0.232	1.15	MLC1	Megalencephalic leukoencephalopathy with subcortical cysts 1	0.208	2.52
NR1D1	Nuclear receptor subfamily 1, group D, member 1	0.233	-1.28	GABRA5	Gamma-aminobutyric acid (GABA) A receptor, alpha 5	0.213	-1.78

Top 10 changes in gene expression levels between treated (10 μ M amphetamine, 10 μ M cocaine, 1 mM lithium and 5 mM sodium valproate) and untreated conditions measured using qPCR arrays (Human Mood Disorder 96 StellarRayTM) and Global Pattern Recognition (GPR) statistical analysis. Fold change values are represented as treated conditions normalised to the drug vehicle. Bold font indicates significant changes in gene expression, $p < 0.05$.

Table 3. Predicted NRSF regulation of genes affecting mood.

Gene	Locus	Strand	NRSF site	Size (Bp)	Position
ACE	chr17:61554422-61575741	+	chr17:61553914-61554174	260	-508
ACE	chr17:61554422-61575741	+	chr17:61554504-61554774	270	82
ACE	chr17:61554422-61575741	+	chr17:61556270-61556594	324	1848
ACE	chr17:61554422-61575741	+	chr17:61557174-61557444	270	2752
ACE	chr17:61554422-61575741	+	chr17:61558309-61558579	270	3887
ADRBK2	chr22:25960861-26125258	+	chr22:25961290-25961560	270	429
ADRBK2	chr22:25960861-26125258	+	chr22:26052841-26053085	244	91980
ADRBK2	chr22:25960861-26125258	+	chr22:26097050-26097320	270	136189
ARNTL	chr11:13277734-13387266	+	chr11:13283216-13283586	370	5482
ARNTL	chr11:13277734-13387266	+	chr11:13298458-13299341	883	20724
ARNTL	chr11:13277734-13387266	+	chr11:13310624-13311040	416	32890
ARNTL	chr11:13277734-13387266	+	chr11:13312905-13313275	370	35171
ARNTL	chr11:13277734-13387266	+	chr11:13351630-13351900	270	73896
ARNTL	chr11:13277734-13387266	+	chr11:13361071-13361575	504	83337
ARNTL	chr11:13277734-13387266	+	chr11:13364729-13364973	244	86995
ARNTL	chr11:13277734-13387266	+	chr11:13365612-13366116	504	87878
BCR	chr22:23522552-23660224	+	chr22:23525622-23525892	270	3070
BCR	chr22:23522552-23660224	+	chr22:23546679-23546949	270	24127
BCR	chr22:23522552-23660224	+	chr22:23562075-23562399	324	39523
BCR	chr22:23522552-23660224	+	chr22:23566052-23566322	270	43500
BCR	chr22:23522552-23660224	+	chr22:23591914-23592184	270	69362
BCR	chr22:23522552-23660224	+	chr22:23624008-23624332	324	101456
BCR	chr22:23522552-23660224	+	chr22:23647903-23648174	271	125351
BCR	chr22:23522552-23660224	+	chr22:23651156-23651400	244	128604
BDNF	chr11:27676442-27743605	-	chr11:27667673-27667943	270	-8499
BDNF	chr11:27676442-27743605	-	chr11:27671454-27671716	262	-4726
BDNF	chr11:27676442-27743605	-	chr11:27680076-27680346	270	63259
BDNF	chr11:27676442-27743605	-	chr11:27721240-27721484	244	22121
BDNF	chr11:27676442-27743605	-	chr11:27723005-27723329	324	20276
BDNF	chr11:27676442-27743605	-	chr11:27739843-27740167	324	3438
BDNF	chr11:27676442-27743605	-	chr11:27740692-27741122	430	2483
BDNF	chr11:27676442-27743605	-	chr11:27741795-27742502	707	1103
BDNF	chr11:27676442-27743605	-	chr11:27742701-27743071	370	534
BDNF	chr11:27676442-27743605	-	chr11:27743607-27744258	651	+2
BDNF	chr11:27676442-27743605	-	chr11:27744566-27744890	324	+961
CASP8	chr2:202098166-202152434	+	chr2:202096900-202097280	380	-1266
CASP8	chr2:202098166-202152434	+	chr2:202098061-202098441	380	-105
CASP8	chr2:202098166-202152434	+	chr2:202122713-202123093	380	24547
CRH	chr8:67088612-67090846	-	chr8:67089099-67090281	1182	565
CRH	chr8:67088612-67090846	-	chr8:67090287-67090659	372	187
CRH	chr8:67088612-67090846	-	chr8:67090956-67091280	324	+110
CRH	chr8:67088612-67090846	-	chr8:67091915-67092285	370	+1069
CRH	chr8:67088612-67090846	-	chr8:67098519-67098889	370	+7673
DISC1	chr1:231762561-232177019	+	chr1:231795960-231796330	370	33399
DISC1	chr1:231762561-232177019	+	chr1:231814930-231815200	270	52369
DISC1	chr1:231762561-232177019	+	chr1:231925791-231926295	504	163230
DISC1	chr1:231762561-232177019	+	chr1:231963016-231963520	504	200455
DISC1	chr1:231762561-232177019	+	chr1:231964053-231964309	256	201492
DISC1	chr1:231762561-232177019	+	chr1:232067746-232067990	244	305185
DISC1	chr1:231762561-232177019	+	chr1:232148522-232148892	370	385961
DRD3	chr3:113847557-113918254	-	chr3:113871366-113871690	324	46564
DRD3	chr3:113847557-113918254	-	chr3:113874262-113874642	380	43612
DRD3	chr3:113847557-113918254	-	chr3:113897607-113898013	406	20241
DRD3	chr3:113847557-113918254	-	chr3:113898443-113898813	370	19441
DRD4	chr11:637305-640705	+	chr11:640330-640654	324	3025
DTNBP1	chr6:15523032-15663289	-	chr6:15552018-15552288	270	111001
DTNBP1	chr6:15523032-15663289	-	chr6:15621994-15622224	230	41065

DTNBP1	chr6:15523032-15663289	-	chr6:15662506-15662830	324	459
FKBP5	chr6:35541362-35696397	-	chr6:35656504-35656848	344	39549
FKBP5	chr6:35541362-35696397	-	chr6:35687515-35687759	244	8638
FKBP5	chr6:35541362-35696397	-	chr6:35695292-35695562	270	835
FKBP5	chr6:35541362-35696397	-	chr6:35695873-35696103	230	294
FKBP5	chr6:35541362-35696397	-	chr6:35699743-35700105	362	-3346
FOS	chr14:75745481-75748937	+	chr14:75743830-75744074	244	-1651
FOS	chr14:75745481-75748937	+	chr14:75745296-75745800	504	-185
GABRA5	chr15:27111866-27194357	+	chr15:27110041-27110545	504	-1825
GABRA5	chr15:27111866-27194357	+	chr15:27111625-27112129	504	-241
GAD1	chr2:171673200-171717659	+	chr2:171670663-171671101	438	-2537
GAD1	chr2:171673200-171717659	+	chr2:171671290-171671546	256	-1910
GAD1	chr2:171673200-171717659	+	chr2:171672190-171672567	377	-1010
GAD1	chr2:171673200-171717659	+	chr2:171679546-171679776	230	6346
GAD1	chr2:171673200-171717659	+	chr2:171701873-171702253	380	28673
GRIK3	chr1:37261128-37499844	-	chr1:37269486-37269856	370	229988
GRIK3	chr1:37261128-37499844	-	chr1:37301874-37302144	270	197700
GRIK3	chr1:37261128-37499844	-	chr1:37329834-37330078	244	169766
GRIK3	chr1:37261128-37499844	-	chr1:37331752-37332256	504	167588
GRIK3	chr1:37261128-37499844	-	chr1:37332540-37332784	244	167060
GRIK3	chr1:37261128-37499844	-	chr1:37388506-37388750	244	111094
GRIK3	chr1:37261128-37499844	-	chr1:37389788-37390253	465	109591
GRIK3	chr1:37261128-37499844	-	chr1:37411488-37411732	244	88112
GRIK3	chr1:37261128-37499844	-	chr1:37431706-37432281	575	67563
GRIK3	chr1:37261128-37499844	-	chr1:37486267-37486654	387	13190
GRIK3	chr1:37261128-37499844	-	chr1:37494616-37494860	244	4984
GRIK3	chr1:37261128-37499844	-	chr1:37504779-37505043	264	-4935
GRM3	chr7:86273230-86494192	+	chr7:86290343-86290599	256	17113
GRM3	chr7:86273230-86494192	+	chr7:86322086-86322456	370	48856
GRM3	chr7:86273230-86494192	+	chr7:86476174-86476554	380	202944
GRM3	chr7:86273230-86494192	+	chr7:86497476-86497720	244	+3284
JUN	chr1:59246463-59249785	-	chr1:59249472-59249885	413	-100
MAG	chr19:35782989-35820133	+	chr19:35796870-35797100	230	13881
MAG	chr19:35782989-35820133	+	chr19:35809956-35810280	324	26967
MAOA	chrX:43,515,409-43,606,068	+	-	-	-
MLC1	chr22:50,497,820-50,523,781	-	-	-	-
MOBP	chr3:39543557-39567857	+	chr3:39540121-39540386	265	-3436
MOBP	chr3:39543557-39567857	+	chr3:39558349-39558719	370	14792
MOBP	chr3:39543557-39567857	+	chr3:39574318-39574698	380	+6461
MTHFR	chr1:11845787-11866160	-	chr1:11845214-11845454	240	+573
MTHFR	chr1:11845787-11866160	-	chr1:11850982-11851306	324	14854
MTHFR	chr1:11845787-11866160	-	chr1:11856563-11856793	230	9367
MTHFR	chr1:11845787-11866160	-	chr1:11857775-11857960	185	8200
MTHFR	chr1:11845787-11866160	-	chr1:11858618-11858699	81	7461
MTHFR	chr1:11845787-11866160	-	chr1:11863764-11864034	270	2126
MTHFR	chr1:11845787-11866160	-	chr1:11865502-11865882	380	278
MTHFR	chr1:11845787-11866160	-	chr1:11866038-11866425	387	-265
NAPG	chr18:10525873-1052766	+	chr18:10525815-10526242	427	-58
NCAM1	chr11:112831969-113092626	+	chr11:112831909-112832179	270	-60
NCAM1	chr11:112831969-113092626	+	chr11:112977293-112977549	256	145324
NCAM1	chr11:112831969-113092626	+	chr11:113008930-113009200	270	176961
NCAM1	chr11:112831969-113092626	+	chr11:113011853-113012123	270	179884
NCAM1	chr11:112831969-113092626	+	chr11:113023160-113023664	504	191191
NCAM1	chr11:112831969-113092626	+	chr11:113074175-113074445	270	242206
NR1D1	chr17:38249037-38256973	-	chr17:38244467-38244847	380	+4570
NR1D1	chr17:38249037-38256973	-	chr17:38254215-38254595	380	2378
NR1D1	chr17:38249037-38256973	-	chr17:38255228-38255666	438	1307
NR1D1	chr17:38249037-38256973	-	chr17:38256685-38257094	409	-121
NR1D1	chr17:38249037-38256973	-	chr17:38257324-38257828	504	-351
NR1D1	chr17:38249037-38256973	-	chr17:38264445-38264769	324	-7472

NR3C1	chr5:142657496-142783254	-	chr5:142784785-142785394	609	-2140
NRG1	chr8:31496911-32622558	+	chr8:31499444-31499814	370	2533
NRG1	chr8:31496911-32622558	+	chr8:31612484-31612740	256	115573
NRG1	chr8:31496911-32622558	+	chr8:31629195-31629565	370	132284
NRG1	chr8:31496911-32622558	+	chr8:31652781-31653242	461	155870
NRG1	chr8:31496911-32622558	+	chr8:31691004-31691508	504	194093
NRG1	chr8:31496911-32622558	+	chr8:31817830-31818086	256	320919
NRG1	chr8:31496911-32622558	+	chr8:31896212-31896582	370	399301
NRG1	chr8:31496911-32622558	+	chr8:32084240-32084744	504	587329
NRG1	chr8:31496911-32622558	+	chr8:32122327-32122831	504	625416
NRG1	chr8:31496911-32622558	+	chr8:32189091-32189595	504	692180
NRG1	chr8:31496911-32622558	+	chr8:32191794-32192298	504	694883
NRG1	chr8:31496911-32622558	+	chr8:32200953-32201685	732	704042
NRG1	chr8:31496911-32622558	+	chr8:32245491-32245735	244	748580
NRG1	chr8:31496911-32622558	+	chr8:32276508-32276752	244	779597
NRG1	chr8:31496911-32622558	+	chr8:32284202-32284706	504	787291
NRG1	chr8:31496911-32622558	+	chr8:32392615-32392985	370	895704
NRG1	chr8:31496911-32622558	+	chr8:32405958-32406282	324	909047
NRG1	chr8:31496911-32622558	+	chr8:32406492-32406892	400	909581
NRG1	chr8:31496911-32622558	+	chr8:32411341-32411845	504	914430
NRG1	chr8:31496911-32622558	+	chr8:32487206-32487506	300	990295
NRG1	chr8:31496911-32622558	+	chr8:32488853-32489109	256	991942
NRG1	chr8:31496911-32622558	+	chr8:32503654-32504024	370	1006743
NRG1	chr8:31496911-32622558	+	chr8:32546371-32546746	375	1049460
NRG1	chr8:31496911-32622558	+	chr8:32572641-32573145	504	1075730
NRG1	chr8:31496911-32622558	+	chr8:32581201-32581705	504	1084290
NRG1	chr8:31496911-32622558	+	chr8:32582687-32583047	360	1085776
PAFAH1B3	chr19:42801185-42806952	-	chr19:42806435-42806939	504	-13
PER3	chr1:7844714-7905237	+	~14 Kb upstream of 5'UTR	-	-
PDLIM5	chr4:95373038-95509370	+	chr4:95372903-95373283	380	-135
PDLIM5	chr4:95373038-95509370	+	chr4:95406777-95407007	230	33739
PDLIM5	chr4:95373038-95509370	+	chr4:95418920-95419164	244	45882
PDLIM5	chr4:95373038-95509370	+	chr4:95455973-95456203	230	82935
PDLIM5	chr4:95373038-95509370	+	chr4:95456267-95456511	244	83229
PDLIM5	chr4:95373038-95509370	+	chr4:95471601-95471831	230	98563
PDLIM5	chr4:95373038-95509370	+	chr4:95499407-95499663	256	126369
RELN	chr7:103112231-103629963	-	chr7:103127865-103128245	380	501718
RELN	chr7:103112231-103629963	-	chr7:103276613-103276992	379	352971
RELN	chr7:103112231-103629963	-	chr7:103297949-103298179	230	331784
RELN	chr7:103112231-103629963	-	chr7:103301028-103301258	230	328705
RELN	chr7:103112231-103629963	-	chr7:103354935-103355205	270	274758
RELN	chr7:103112231-103629963	-	chr7:103438111-103438481	370	191482
RELN	chr7:103112231-103629963	-	chr7:103451010-103451107	97	178856
RELN	chr7:103112231-103629963	-	chr7:103484281-103484449	168	145514
RELN	chr7:103112231-103629963	-	chr7:103491745-103492249	504	137714
RELN	chr7:103112231-103629963	-	chr7:103559848-103560078	230	69885
RELN	chr7:103112231-103629963	-	chr7:103580845-103581215	370	48748
RELN	chr7:103112231-103629963	-	chr7:103636658-103636861	203	-6898
RFX4	chr12:106976685-107156582	+	chr12:106975282-106975646	364	-1403
RFX4	chr12:106976685-107156582	+	chr12:106975776-106976119	343	-909
RFX4	chr12:106976685-107156582	+	chr12:107147300-107147544	244	170615
RGS4	chr1:163038396-163046592	+	chr1:163039054-163039341	287	658
SLC12A6	chr15:34522197-34630265	-	chr15:34516950-34517512	562	+5247
SLC12A6	chr15:34522197-34630265	-	chr15:34610582-34611086	504	19179
SLC12A6	chr15:34522197-34630265	-	chr15:34630069-34630393	324	-128
SLC12A6	chr15:34522197-34630265	-	chr15:34634991-34635543	552	-4726
SLC6A2	chr16:55689542-55737700	+	chr16:55686047-55686317	270	-3495
SLC6A2	chr16:55689542-55737700	+	chr16:55689638-55689908	270	96
SLC6A2	chr16:55689542-55737700	+	chr16:55690575-55690845	270	1033
SLC6A2	chr16:55689542-55737700	+	chr16:55693927-55694197	270	4385
SLC6A2	chr16:55689542-55737700	+	chr16:55695818-55696088	270	6276

SLC6A2	chr16:55689542-55737700	+	chr16:55696686-55696956	270	7144
SLC6A2	chr16:55689542-55737700	+	chr16:55744402-55744761	359	+7061
SLC6A2	chr16:55689542-55737700	+	chr16:55746277-55746521	244	+8821
SLC6A4	chr17:28,523,378-28,562,954	-	-	-	-
SULT1A1	chr16:28616908-28634907	-	chr16:28621167-28621407	240	13500
TF	chr3:133419211-133497850	+	chr3:133461483-133461863	380	42272
TF	chr3:133419211-133497850	+	chr3:133465027-133465407	380	45816
TF	chr3:133419211-133497850	+	chr3:133472690-133472920	230	53479
TIMELESS	chr12:56810157-56843200	-	chr12:56811537-56811907	370	31293
TIMELESS	chr12:56810157-56843200	-	chr12:56842752-56843263	511	-63
TPH2	chr12:72332626-72426221	+	chr12:72332400-72332889	489	-226
TPH2	chr12:72332626-72426221	+	chr12:72374868-72375372	504	42242
TPH2	chr12:72332626-72426221	+	chr12:72410895-72411165	270	78269
XBP1	chr22:29190548-29196560	-	chr22:29196394-29196960	566	-400
XBP1	chr22:29190548-29196560	-	chr22:29198252-29198482	230	-1922

NRSF binding sites over top 10 affected genes across all drug treatments from Transcription Factor ChIP-seq from ENCODE version 4. Bold font indicates genes significantly affected by drug challenge. Negative and positive values under *Position* represent the location of the NRSF site upstream of the gene transcriptional start site and downstream of the 3'UTR, respectively. Values not assigned +/- represent binding sites within the gene sequence. For genes with multiple transcripts, binding site positions are with respect to the largest isoform.

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*Conflict of Interest

Conflicts of interest

The authors report no conflicts of interest

*Role of the Funding Source

Role of funding source

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Contribution of authors

Warburton was involved in experimental design, data acquisition, analysis of data and manuscript preparation. Savage and Myers were involved in experimental design and data acquisition. Peeney was involved in analysis of data. Quinn and Bubb were involved in experimental design, analysis of data and manuscript preparation. All authors have approved the final manuscript.

Highlights

- In vitro affects of mood modifying drugs on the transcriptome
- qPCR arrays as a cost effective approach to address drug specific expression change
- Global Pattern Recognition and pathway analysis of gene expression data
- NRSF and oestrogen receptor signalling in response to mood stabilisers
- Model can be used for comparing multiple drug regimes or in initial drug screening