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Candidate genes, pathways and mechanisms for bipolar (manic–depressive) and related disorders: an expanded convergent functional genomics approach

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Identifying genes for bipolar mood disorders through classic genetics has proven difficult. Here, we present a comprehensive convergent approach that translationally integrates brain gene expression data from a relevant pharmacogenomic mouse model (involving treatments with a stimulant—methamphetamine, and a mood stabilizer—valproate), with human data (linkage loci from human genetic studies, changes in postmortem brains from patients), as a bayesian strategy of crossvalidating findings. Topping the list of candidate genes, we have DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa) located at 17q12, PENK (preproenkephalin) located at 8q12.1, and TAC1 (tachykinin 1, substance P) located at 7q21.3. These data suggest that more primitive molecular mechanisms involved in pleasure and pain may have been recruited by evolution to play a role in higher mental functions such as mood. The analysis also revealed other high-probability candidates genes (neurogenesis, neurotrophic, neurotransmitter, signal transduction, circadian, synaptic, and myelin related), pathways and mechanisms of likely importance in pathophysiology.
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Bipolar (manic–depressive) disorders are characterized by alternating episodes of elevated and depressed mood. Severe episodes have psychotic features similar to some of the symptoms of schizophrenia, that is, positive psychotic symptoms (hallucinations, delusions) in mania, and negative psychotic symptoms (lack of motivation, psychomotor retardation) in depression. The genetic basis of bipolar disorder and schizophrenia are well documented, with an incidence of about 1% in the general population. Having a first-degree relative with the illness increases the likelihood of developing the illness by about 10-fold. Traditionally, linkage analysis and positional cloning approaches have been used to try to identify the genes involved. This has led to the identification of a series of loci in the genome that exhibit linkage with the illness. Several of these loci are identified in both bipolar disorder and schizophrenia studies, suggesting the possibility of shared genes between these disorders.[1–3] As these disorders are likely polygenic, non-Mendelian with variable penetrance, and the clinical phenotypes are complex, there has been limited success so far in terms of reproducible findings. The linkage peaks supported by the most recent meta-analyses of genome scan data[4,5] are fairly broad, with hundreds of genes in each peak. A method of prioritizing candidate genes for individual analysis of association with illness is critical. We have previously described initial proof of principle for one such approach that we have termed Convergent Functional Genomics.[6] The approach integrates gene expression data from a relevant animal model with human linkage data, as a way of crossvalidating findings and coming up with a short list of high-probability candidate genes that deserve individual scrutiny in a prioritized manner. Here, we report the first comprehensive analysis using an expanded Convergent Functional Genomics approach as a way of unraveling the genetic code of bipolar and related disorders.

Single-dose methamphetamine treatment in humans and animals mimics many of the behavioral signs and symptoms of bipolar disorder—mania features during the activation phase (elevated mood, increased energy, hyperlocomotion, perseverative behavior, hypersexuality), and depressive features during the withdrawal phase (low mood, low energy, decreased locomotion, passivity, anhedonia)[6–12] (Figure 1a). Amphetamine challenge led to a significantly greater behavioral response in euthymic bipolar disorder patients than in healthy subjects,[13]

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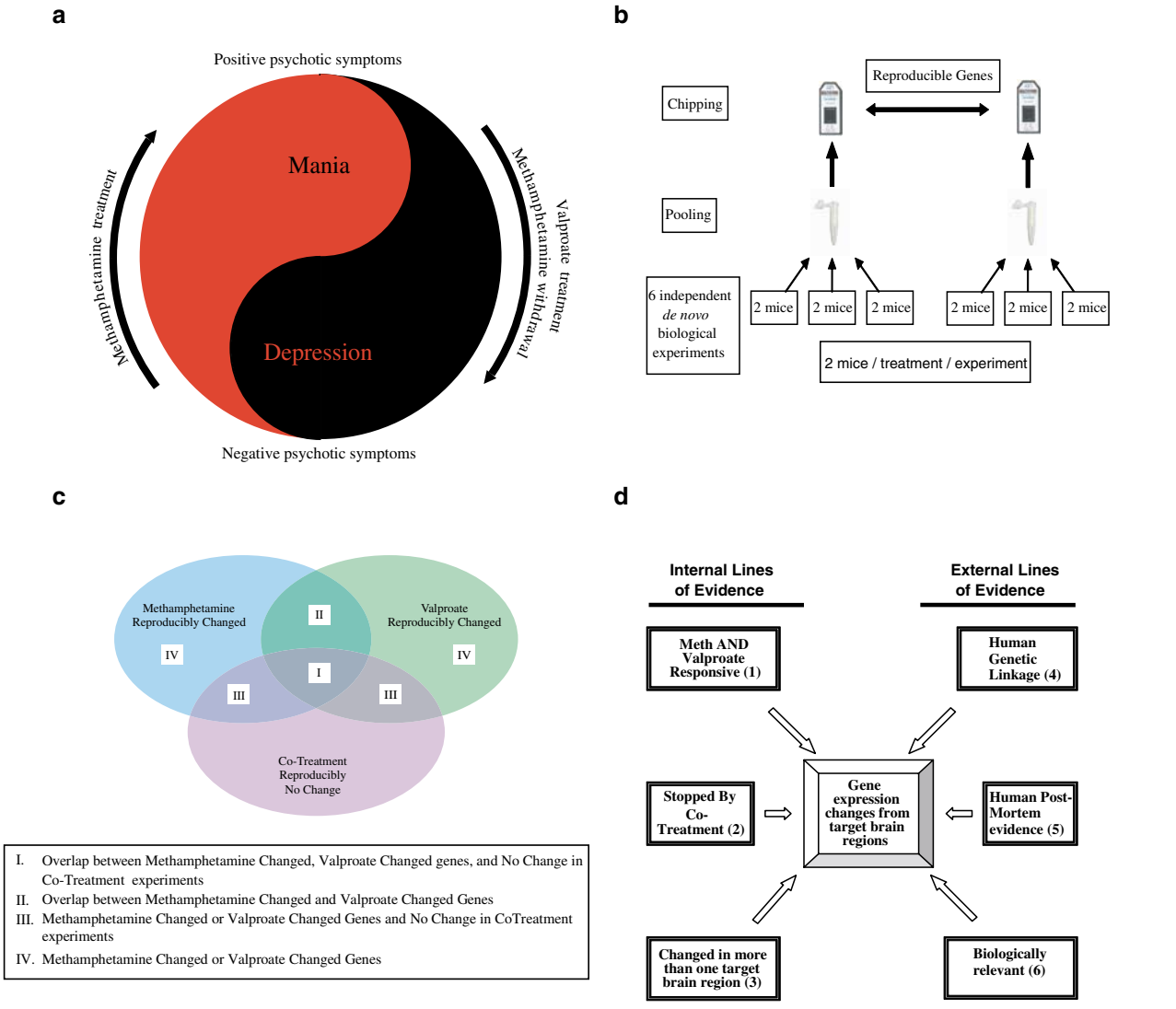
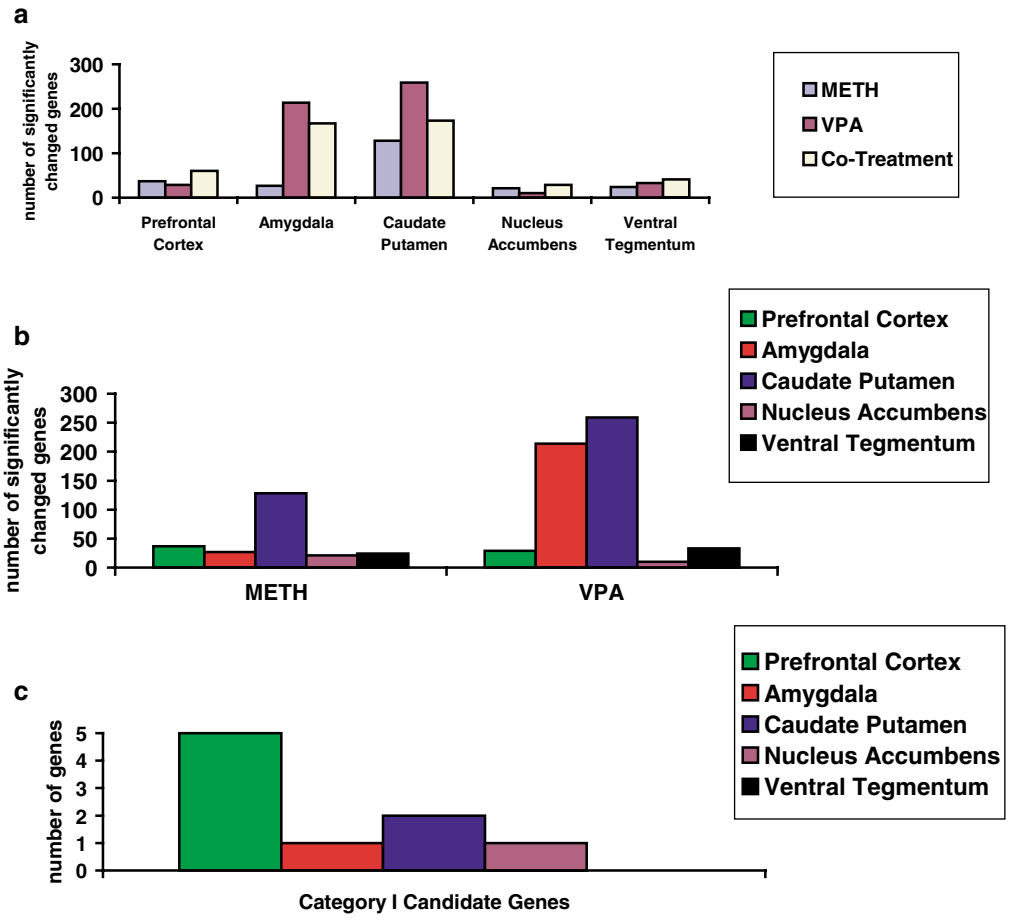


Figure 1 Design of experiments and data analysis: (a) pharmacological treatment paradigm, (b) experimental design, (c) Venn diagram categorizing genes changed by the various drug treatments, and their classification into Categories I–IV, (d) multiple converging independent internal and external lines of evidence for crossvalidation of findings.

suggesting that amphetamines act on at least some of the pathways involved in bipolar disorder.

Valproate, an anticonvulsant mood stabilizer, is one of the current mainstays of treatment for bipolar disorder, and has been shown to interfere with and treat the development of full-blown manic symptomatology. For mania, the spectrum of efficacy of valproate is broader than for other mood stabilizers.[14]

In essence, in our approval we are using drug effects on gene expression as tools to tag genes that may have pathophysiological relevance. Changes of gene expression in response to each of the two drugs, methamphetamine and valproate might be of interest in and of themselves, and in terms of candidate gene generation and convergent functional genomics. However, not all genes that show changes in expression in response to either of the drugs are necessarily germane to the pathophysiology of bipolar and related disorders. It is likely that some of them have to do with other effects of the drugs, and with their individual side effects. We hence used three internal criteria for crossvalidation (Figure 1d). We reasoned, first, that the genes that changed in expression in response to both drugs are more likely to be core to the pathophysiological process, and are higher probability candidate genes. Second, cotreatment with the two drugs, one a bipolar inducing, and the other one a bipolar disorder-treating drug, could arguably show interference effects (Figure 2), and some genes that would be changed by single drug treatment would be ''nipped in the bud'' and not show changes in expression by cotreatment. Those genes would also



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Brain Region \ Category	I	II	Meth III	VPA III	Meth IV	VPA IV	Totals
Prefrontal Cortex	5	0	12	14	20	10	61
Amygdala	1	9	8	68	9	136	231
Caudate Putamen	2	23	28	25	75	209	362
Nucleus Accumbens	1	3	9	0	8	6	27
Ventral Tegmentum	0	8	0	4	16	21	49

Figure 2 Number of genes reproducibly changed: METH—methamphetamine; VPA—valproate. (a) Comparative effects of methamphetamine, valproate, and cotreatment with both drugs in different target brain regions, showing interference effects of cotreatment. (b) Brain region-specific differences of drug treatment with methamphetamine and valproate on gene expression. (c) Distribution of Category I candidate genes across brain regions. (d) Number of reproducibly changed genes in Categories I–IV.

be deemed higher probability candidate genes than genes that still change during cotreatment. Third, we comprehensively surveyed gene expression changes across five different brain regions (prefrontal cortex (PFC), amygdala (AMY), caudate-putamen (CP), nucleus accumbens (NA), and ventral tegmentum (VT)) that have shown evidence, in human imaging, human postmortem, or animal studies, of being potentially implicated in bipolar and related disorders pathophysiology.[13,15–17] We reasoned that if a gene is changed in more than one of these brain regions, it may be a higher probability candidate gene compared to genes that are changed in a single region.

As external crossvalidators, we used three criteria (Figure 1d). First, does the gene map to a linkage locus that has been reported to be associated with bipolar disorder, or more broadly to schizophrenia or depression? For this, we used our earlier published

criterion, which is that the gene had to map to within 10 centimorgans (cM) of a marker for which evidence for linkage had been reported in at least one published study.[6] Second, is there any human postmortem data showing changes in expression of that gene in brains from patients with bipolar disorder, schizophrenia, depression, or at the least other brain conditions that impact mood and cognition, such as substance abuse, Alzheimer or mental retardation? Third, does the gene have a known biological function that is relevant to the pathophysiology of bipolar and related disorders, or more broadly to neuronal activity? These external criteria suffer from the obvious drawback of being constrained by what has been published so far, limiting novelty, and to the inherent biases and limitations of those particular lines of work (ie relatively more postmortem data to date available for schizophrenia than for bipolar disorders or depression). Moreover, these external criteria are arguably broad, and may benefit from future parsing. One argument in their favor is the emerging appreciation of the modular endophenotypic overlap between bipolar disorders, schizophrenia and depression,[18,19] and the neuronal hyperactivity, respectively hypoactivity, associated with different subtypes.

For each gene in our data sets, using the three internal and three external crossvalidators described above (Figure 1d), and assigning a generic score of 1 for each criterion, an empirical tabulation of independent lines of evidence was generated. According to bayesian theory, an optimal estimate results from combining prior information with new evidence.[20] While we cannot exclude that some of the candidate genes we have identified are false positives due to potential biological or technical limitations of the methodology and approach we employed, the higher the number of independent lines of evidence, the lower the likelihood of that being the case.

Our approach identifies an extensive series of candidate genes, some of which have already been reported using various related treatments or paradigms,[3,12,21–24] and thus serve as positive controls, as well as many that are novel. Moreover, the coalescence of the candidate genes into pathways and mechanisms is of particular importance and opens new directions. Last but not the least, as per our earlier formulation that 'genes that change together (may) act together',[6] the coexpression data sets we have generated in various brain regions offer testable hypothesis for transcriptional coregulation, and for epistatic interactions among the corresponding loci.

Materials and methods

Methamphetamine and valproate treatments in mice
All experiments were performed with male C57/BL6 mice, 8–12 weeks of age, obtained from Jackson Laboratories (Bar Harbor, ME, USA), and acclimated for at least 2 weeks in our animal facility (VASDHS Veterinary Medical Unit) prior to any experimental manipulation. Mice were treated by intraperitoneal injection with either single-dose saline, methamphetamine (10 mg/kg), valproate (200 mg/kg), or a combination of methamphetamine and valproate (10 mg/kg/200 mg/kg). Six independent *de novo* biological experiments were performed at different times. Each experiment consisted of two mice per treatment condition, for a total of 12 mice per condition across the six experiments (Figure 1b).

Behavioral studies and analysis
Locomotor activity was measured immediately after drug administration and again 24 h later. At the beginning of the test session, each mouse was placed in an enclosure with predefined areas, that is, center area, corner area, and wall area. The movements of the mice were recorded for 30 min, with data being stored in six 5-min blocks. The spatial scaling exponent, *D*, or spatial *D*, a measure of the geometric patterns of locomotor activity, was quantified, as described in detail elsewhere.[25] Briefly, spatial *D* is a measure of the nonlinear nature of an animal's locomotor movement and is quantified on a scale from 1 to 2, with the lower bound indicating extremely linear movement and 2 representing highly nonlinear locomotor movement.

Microdissection
At 24 h after drug administration, following the 24-h time-point behavioral test, the brains of the mice were harvested and stereotactically sliced. Specific brain regions—PFC, AMY, CP, NA, and VT—were microdissected. Tissue samples were flash frozen in liquid nitrogen and stored in −80°C until future processing for RNA extraction and gene expression analysis.

Microarrays
We used Murine Genome U74A and Bv2 oligonucleotide arrays (Affymetrix, Santa Clara, CA, USA) as described at http://www.affymetrix.com/products/arrays/specific/mgu74.affx. The U74Av2 chip contains approximately 6000 genes and 6000 ESTs, while the U74Bv2 contains approximately 12 000 ESTs. Microarrays used in each independent experiment were derived from the same manufacturing lot. (http://www.affymetrix.com/support/downloads/manuals/expression_s2_manual.pdf).

Microarray experiments
Standard techniques were used to obtain RNA (syringe homogenization in RLT buffer) and to purify the RNA (RNeasy mini kit (Qiagen, Valencia, CA, USA) from dissected mouse brain regions. The quality of the total RNA was confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The quantity and quality of total RNA was also independently assessed by 260 nm UV absorption and by 260/280 ratios, respectively (Beckman DU 640B spectrophotometer (Beckman Coulter, Fullerton, CA,

USA)). Starting material of total RNA amplification and labeling reactions was kept consistent within each independent microarray experiment.

Total RNA extracted from tissue samples was pooled from the first three independent biological experiments (six mice per treatment group) and used for the first microarray experiment, and from the final three independent biological experiments (six mice per treatment group) for the second microarray experiment. The microarray experiments were conducted independently, at different times. A T7-linked oligo(dT) primer was used to reverse transcribe the messenger RNA. Biotin-labeled cDNA was generated using the Enzo BioArray High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY, USA). The quality and quantity of the cDNA was assessed using the same methods (described above) that were used to assess the total RNA. The amount of cDNA used to prepare the hybridization cocktail was kept constant intraexperiment. Samples were hybridized at 45° for 16 h under constant rotation. Arrays were washed and stained using the Affymetrix Fluidics Station 400 and scanned using the Affymetrix GeneArray Scanner 2500. All sample labeling, hybridization, staining and scanning procedures were carried out as per manufacturer's recommendations.

Quality control

All arrays were scaled to a target intensity of 2500 using the Affymetrix MASv 5.0 array analysis software. Quality control measures including 3′/5′ ratios for GAPDH and beta-actin, scaling factors, background, and *Q*-values were within acceptable limits (detailed information can be found at http://www.neurophenomics.info/high_probability_candidate_genes.htm.

Microarray data analysis

Data analysis was performed using the Affymetrix Microarray Suite 5.0 software (MAS v5.0). The calculation of the ratio between perfect match (PM) to mismatch (MM) (PM/MM ratio) was used to define transcripts as present (P), marginal (M), or absent (A). We used the default settings provided by Affymetrix for this determination. A comparison analysis was performed for each drug treatment, using its corresponding saline treatment as the baseline. 'Signal,' 'Detection,' 'Signal Log Ratio,' 'Change,' and 'Change *P*-value,' were obtained from this analysis. Fold change was calculated from the signal log ratio. Only transcripts that were called Present in at least one of the two samples (saline or drug) intraexperiment, and that were reproducibly changed in the same direction across independent experiments, were analyzed further.

Complete data sets with all raw data values for each pooled sample and multiple probe level analysis results (MIAME report) can be found at http://www.neurophenomics.info/high_probability_candidate_genes.htm.

Gene identification

The Affymetrix Interactive Query feature was used to verify each gene name from the probe-set information. In the case of ESTs where the Affymetrix website did not identify a known gene by name, a National Center for Biotechnology Information (NCBI) (Bethesda, MD, USA) Blast analysis was carried out, to identify the closest known mouse gene existing in the database (the highest homology mouse gene, at the top of the Blast list of homologues), and then using GeneCards (Weizmann Institute, Rehovot, Israel) to identify the information about the human homologue. Where no known mouse gene was at the top of the BLAST homology list, the construct was labeled as just 'EST' in our data sets and tables.

Biological and postmortem convergence

Information about our candidate genes was obtained using GeneCards, as well as database searches using PubMed (http://www.ncbi.nlm.nih.gov/PubMed/) and the various combinations of key words (gene name, brain, human, bipolar, schizophrenia, depression, suicide, postmortem). Genes were deemed to have biological convergence if their known biological function was relevant to the pathophysiology of bipolar and related disorders in human or animal models. Postmortem convergence was deemed to occur for a gene if there were published reports of human postmortem data showing changes in expression of that gene in brains from patients with bipolar disorder, schizophrenia, depression, or other brain disorders that impact mood and cognition.

Genetic linkage convergence

To designate convergences for a particular gene, the gene had to map to within 10 cM of a microsatellite marker for which at least suggestive evidence for linkage to bipolar disorder, schizophrenia or depression has been published. The University of Southampton's sequence-based integrated map of the human genome (The Genetic Epidemiology Group, Human Genetics Division, School of Medicine, University of Southampton; http://cedar.genetics.soton.ac.uk/public_html/) was used to obtain cM locations for both genes and markers. The sex-averaged cM value was calculated and used to determine convergence to a particular marker. For markers that were not present in the Southampton database, the Marshfield integrated linkage map (Center for Medical Genetics, Marshfield, WI, USA) was used with the NCBI Map Viewer website to evaluate linkage convergence.

Gene Ontology (GO) analysis

The NetAffx Gene Ontology Mining Tool (Affymetrix, Santa Clara, CA, USA) was employed to categorize the genes in our data sets into functional categories, using the Biological Process ontology branch.

Results

Based on the changes in response to single drug treatment and cotreatment, we divided our data set of reproducibly changed genes into four categories (Figure 1c and Figure 2). Category I includes genes that are changed by both methamphetamine and valproate, and the change is prevented (ie, No Change) by cotreatment with both drugs. Category II includes genes that are changed by both methamphetamine and valproate, but those changes are not prevented by cotreatment. Category III includes genes that are changed by either methamphetamine or valproate, and the change is prevented (No Change) by cotreatment. Category IV includes genes that are changed by one of the drugs only, and the changes are not prevented by cotreatment.

Number of genes

Methamphetamine had the highest number of genes changed in CP, followed by the PFC as a distant second. Valproate had the highest number of genes changed in the CP also, followed closely by the AMY. Nevertheless, a disproportionate number of high-probability, category I genes were in the PFC, consistent with the likely central role of this region in the pathophysiology of bipolar and related disorders (Figure 2).

Top findings

The genes in Categories I and II are shown in Table 1. Figure 3 summarizes the assigned empirical probability score based on the multiple internal and external lines of evidence. It is notable that again, the PFC genes have the highest average score, whereas the other brain regions have lower scores. At the top of our list, with five out of six lines of evidence, we have seven genes: four from the PFC—TAC1, located at 7q21.3;[26,27] PENK, located at 8q12.1;[28] DARPP-32, located at 17q12;[4] and MEF2C (myocyte enhancer factor 2C),located at 5q14.3; two from the CP—CCK (cholecystokinin) located at 3p22–p21.3[5] and TBR1 (T-box brain gene 1) located at 2q24.2;[5] and one from the VT—GLUL (glutamine synthase) located at 1q25.3.[5]

DARPP-32

Notably, five of these top genes are known to interact in a network with DARPP-32 at its core (Figure 5a). DARPP-32 is involved in regulating substance P expression in the striatonigral pathway.[29] Regulation of DARPP-32 phosphorylation is involved in mediating some of the effects exerted by enkephalin on striatal neurons.[30] CCK regulates DARPP-32 phosphorylation in the neostriatum.[31] GLUL indirectly regulates DARPP-32 activity by regulating glutamate metabolism.[32] Moreover, several other genes in our data set, with four out of six lines of evidence or three out of six lines of evidence, are part of the DARPP-32 pathway (Figure 5a). CDK5R1 (cyclin-dependent kinase 5, regulatory subunit 1 (p35)) (Table 1), located at 17q11.2, activates CDK5, which, among other things, modulates dopamine signaling in neurons by phosphorylating DARPP-32.[33] GSK3b (glycogen synthase kinase 3 beta), located at 3q13.3,[34] is a downstream target of the DARPP-32 pathway,[35] and has been implicated in schizophrenia.[36] CAMKK2 (calcium/calmodulin-dependent protein kinase kinase 2)) (Table 1), located at 12q24.31;[37] GRM3 (glutamate receptor, metabotropic 3) (Table 2), located at 7q21.12;[26] GRIK5 (glutamate receptor, ionotropic, kainate 5) (Table 3),located at 19q13.2;[38] and GAT3 (GABA transporter 3) (Table 1), located at 3p25.3, all have potential direct or indirect inhibitory effects on the DARPP-32 activity[39–41] (Figure 5a). As a caveat, it should be noted that most of the above inter-relationships were inferred from work focused on striatal function. However, it is reasonable to assume that similar inter-relationships might be functional in other dopaminergic neuronal populations, such as the meso-cortical dopamine pathway.[35] Other investigators have previously implicated a majority of the above discussed genes, individually or as part of functional groups, in various biological and genetic contexts germane to the pathophysiology of bipolar and related disorders (Tables 1–3). Our results, identifying these genes as top candidate genes, are thus a strong validation of the heuristic value and internal consistency of the approach we have used. Moreover, they outline networks of potentially coacting genes, and support an important role for the DARPP-32 pathway in bipolar and related disorders.

DARPP-32 has been previously identified as being at the crossroads of the mechanisms of action of various different psychomimetic drugs of abuse.[35] It has also been shown to mediate the stimulant actions of caffeine,[42] of the antidepressant fluoxetine,[43] possible tolerance to alcohol,[44] and progesterone-mediated sexual receptivity.[45] Transgenic mice lacking the DARPP-32 gene displayed deficits in their molecular, electrophysiological, and behavioral response to dopamine, drugs of abuse, and antipsychotic medication.[46] Moreover, ΔARPP-32 has been shown in postmortem studies to be decreased in the PFC of schizophrenic patients.[47]

Pain and mood

The TAC1 gene encodes the neuropeptides substance P and neurokinin A. Mice with TAC1 gene knockout showed decreased depression- and anxiety-related behaviors under a variety of specific behavioral challenges,[48] as well as decreased nociception.[49] Substance P and its pathways, which are implicated in neuropsychiatric syndromes such as mood disorders and somatic symptoms such as pain, are receiving increased attention as drug development targets.[50]

PENK encodes preproenkephalin, which is part of the endogenous opioid system implicated in modulating locomotion, pain perception, and emotional behaviors.[51] Mice lacking preproenkephalin show

Table 1 Categories I and II genes

Mouse Accession Number	Symbol - Description	Meth Fold Change*	VPA Fold Change*	Stopped by Co-Treatment	Multiple Brain Regions	Convergent Functional Genomics	Relevant biological role in brain	Human Postmortem	No. of lines of evidence
PREFRONTAL CORTEX									
Down / Up									
L13171	MEF2C - MADS box transcription enhancer factor 2	0.81 / 0.76	1.23 / 2.46	Yes	**AMY** VPA III 0.76/ 0.44	5q14.3	Yes	Postmitotic neuronal differentiation in the cortex [88]	5/6
Up									
AI839758	DARPP-32 - dopamine- and cAMP-regulated phosphoprotein of 32 kilodaltons	1.62 / 1.74	1.62 / 1.41	Yes		17q12 **BP** [4]	Yes	Decreased PFC of **SZ** [47]	5/6
M55181	PENK - Preproenkephalin 2	1.62 / 2.14	1.74/ 1.62	Yes		8q12.1 **SZ** [28]	Yes	Increased in Substantia Nigra of SZ [89]	5/6
D17584	TAC1 - Tachykinin 1 - substance P	1.52/ 1.74	1.23 / 1.41	Yes		7q21.3 **SZ** [26 27]	Yes	Receptors decreased in orbitofrontal cortex in **MDD** [90] Receptors increased in PFC of **SZ** [91]	5/6
AI852526	GPR88 - G-protein coupled receptor 88	1.74 / 2.46	1.62 / 1.86	Yes		1p21.2	Yes		3/6
AMYGDALA									
Up									
V00835	MT1 - Metallothionein 1	1.51 / 1.51	1.51 / 1.51			16q13	Yes		2/6
AI849207	GORASP2 -Golgi reassembly stacking protein 2	1.41 / 1.32	1.23 / 1.32			2q31.1			1/6
AB007136	PSME1 - Protease (prosome, macropain) 28 subunit, alpha	1.32 / 1.41	1.41 / 1.62			14q11.2			1/6
AA921069	EST	1.41 / 1.62	1.23 / 1.87						1/6
Down									
AW060974	CDK5R1 - Cyclin-dependent kinase 5, regulatory subunit (p35)	0.66 / 0.76	0.76 / 0.57		**CP** VPA III 1.62/ 2.83	17q11.2	Yes	Decreased in brains of opiate addicts [92]	4/6
AI841038	HIP14 - huntingtin interacting protein 14	0.5 / 0.87	0.66/ 0.1			12q21.1	Yes		2/6
AW048171	CLSTN1 - calsyntenin 1	0.66/ 0.81	0.81/ 0.66			1p36.22	Yes		2/6
AI836322	DLC2 - dynein light chain 2	0.62 / 0.47	0.65 / 0.57			17q23.2			1/6
CAUDATE-PUTAMEN									
Up									
U89352	LYPLA1 - Lysophospholipase I	1.41/ 1.62	1.41/ 1.52	Yes		8q11.23	Yes	Abnormal in **SZ**, Alzheimer [93 94]	4/6
D37792	SYT1 - Synaptotegmin 1	1.23/ 8	1.41/ 8		**AMY, VT** VPA IV 0.76/ 0.16, 1.23/ 1.41	12q21.2	Yes	Increased in younger **SZ** [95]	4/6
U19582	CLDN11 - Claudin 11 - Oligodendrocyte specific protein	1.32/ 1.41	1.41/ 1.41			3q26.2 **BP** [75]	Yes	Decreased in the PFC of **SZ** and **BP** [76]	4/6
U81317	MOBP - myelin-associated oligodendrocytic basic protein	1.41/ 3.73	1.52/ 3.73		**VT** VPA IV 1.32/ 1.32	3p22.2 **SZ** [5]	Yes		4/6
AI747899	PITPNB - phosphotidylinositol transfer protein, beta	1.41/ 8.57	1.62/ 8			22q12.1 **SZ** [5]	Yes		3/6
Y13361	RAB7 - Member RAS oncogene family	1.23/ 5.66	1.32/ 5.66		**AMY** VPA IV 0.81/ 0.38	3q21.3	Yes		3/6
AF011379	ADAM10 - A disintegrin and metalloproteinase domain	1.32/ 6.50	1.87/ 8			15q21.3	Yes	Increased in **AD** [96]	3/6
AI837838	TMEFF1 - transmembrane protein	1.52/ 1.41	1.52/ 2			9q31.1 **BP** [97]	Yes		3/6
AB025011	RNF138 - ring finger protein 138	1.23/ 1.52	1.23/ 2.14			18q12.1 **SZ, BP** [98]			2/6
U21855	CNOT7 - CCR4-NOT transcription complex, subunit 7	1.32/ 2.64	1.62/ 3.48			8p22 **BP** [4]			2/6
AW227650	SSR3 - signal sequence receptor, gamma	1.32/ 11.3	1.87/ 13.0			3q25.31 **BP** [99]			2/6
U48896	UGT8 - UDP-glucuronosyltransferase 8	1.32/ 9.19	1.41/ 9.19			4q26	Yes		2/6
D10627	EST - zinc finger transcription factor-like	1.52/ 1.87	1.41/ 2.14						1/6
Down									
U49251	TBR1 - T-box brain gene 1	0.5/ 0.44	0.5/ 0.38		**NA** VPA IV 1.52/ 1.32	2q24.2 SZ [5]	Yes	Increased in **BP** [86]	5/6
X59520	CCK - Cholecystokinin	0.38/ 0.5	0.62/ 0.5		**NA** METH IV 0.76/ 0.47	3p22-p21.3 SZ [5]	Yes	Decreased in **SZ** [100 101]	5/6
AI843866	CAMKK2 - calcium/calmodulin-dependent protein kinase kinase 2, beta	0.66/ 0.87	0.81/ 0.71	Yes		12q24.31 **BP** [37]	Yes		4/6
AI848661	BTBD3 - BTB (POZ) domain containing 3	0.76/ 0.57	0.81/ 0.62		**AMY, PFC** VPA III, IV 0.66/ 0.31, 1.32/ 1.52	20p12.2 **BP** [78]			3/6
AW045893	NCALD - neurocalcin delta	0.76/ 0.54	0.76/ 0.47			8q22.3	Yes		2/6
AW122328	NPTX1 - neuronal pentraxin 1	0.66/ 0.5	0.66/ 0.57			17q25.3	Yes		2/6
AI853550	EST	0.16/ 0.47	0.19/ 0.47						1/6
AI844183	EST	0.76/ 0.76	0.76/ 0.81						1/6
NUCLEUS ACCUMBENS									
Up									
AW120565	GAT3 (SLC6A11) - neurotransmitter transporter, GABA	1.74/ 1.51	1.41/ 1.31	Yes		3p25.3	Yes		3/6
AI837110	PRMT1 - HMT1 hnRNP methyltransferase-like 2	1.86/ 1.74	1.74/ 1.74			19q13.33	Yes		2/6
AW045758	ROXAN - ubiquitous tetratricopeptide containing protein	1.86/ 1.31	1.74/ 1.23			22q13 **BP** [102] **SZ** [5]			2/6
VENTRAL TEGMENTUM									
Down									
AI848384	GLUL - glutamate-ammonia ligase (glutamine synthase)	0.61/ 0.65	0.61/ 0.61		**PFC** METH III 0.71/ 0.81	1q25.3 **SZ** [5]	Yes	Decreased in **SZ** [103] Decreased in **AD** [104]	5/6
AI836414	SRRM2 - serine/arginine repetitive matrix 2	0.70/ 0.75	0.65/ 0.75		**CP** VPA IV 0.81/ 0.66	16p13.3 **BP** [37]			3/6
U70132	PITX2 - paired-like homeodomain transcription factor 2	0.53/ 0.37	0.5/ 0.65			4q25	Yes		2/6
AV325375	MEG3 - maternally expressed gene 3	0.65/ 0.87	0.57/ 0.81			14q32 **BP** [75]			2/6

*Fold changes and *P*-values were calculated using the Affymetrix MAS v5.0 analysis software. All *P*-values were ≤ 0.0028.

Up: upregulated; Down: downregulated; Meth: methamphetamine; VPA: valproate; PFC: prefrontal cortex; AMY: amygdala; CP: caudate putamen; NA: nucleus accumbens; VT: ventral tegmentum; BP: bipolar disorder; SZ: schizophrenia; MDD: major depressive disorder; AD: Alzheimer. Roman numerals in the multiple brain region data column represent the category of the gene.

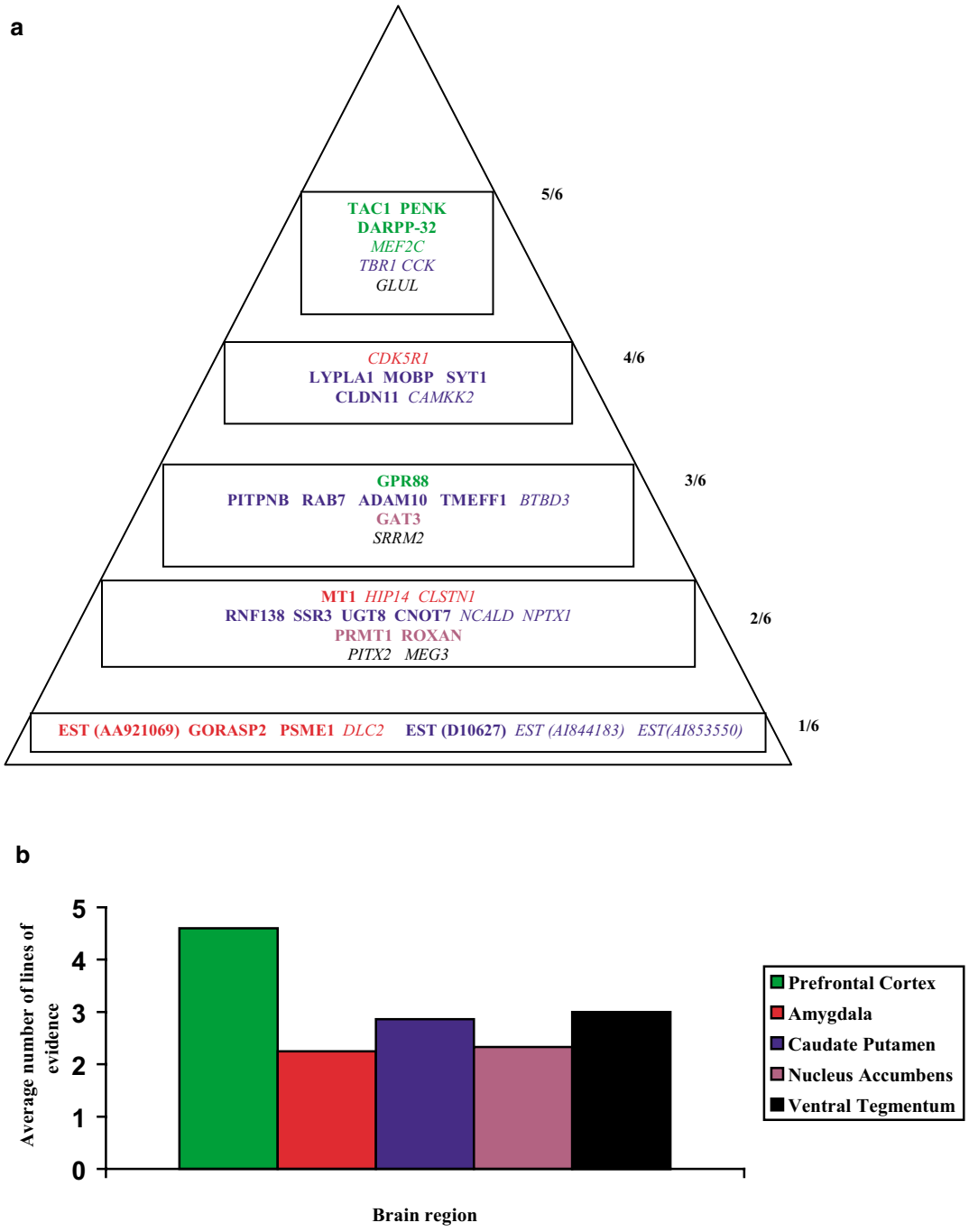

Figure 3 Categories I and II candidate genes. (a) Probability pyramid generated by the tabulation of independent converging lines of evidence. Plain text—increased by methamphetamine. Italics—decreased by methamphetamine. For full description of gene symbols, see Table 1. (b) Comparison of different target brain regions in terms of average number of lines of evidence per candidate gene.

reduced response to the analgesic properties of cannabinoids, as well as reduced withdrawal syndrome to cannabinoids.[52]

Cholecystokinin, originally thought to be confined only to the gastrointestinal tract, is now known to be colocalized in both the gastrointestinal tract and central nervous system, where it has multiple functions. In animal models, levels are increased after neural injury and with opioid administration. This peptide acts as an antiopioid, and has a reciprocal relationship to preproenkephalin.[53] Consistent with that, in our data set we see increased levels of PENK, and decreased levels of CCK (Table 1), albeit in different brain regions.

Table 2 Top category III genes

Mouse Accession Number	Symbol - Description	Brain Region Fold Change	Stopped by Co-Treatment	Multiple Brain Region	Convergent Functional Genomics	Biology	Human postmortem	No. of lines of evidence
	Methamphetamine Changed							
Up								
AV372577	NPY2R - neuropeptide Y receptor Y2	**NA** 1.32/ 1.52	YES		4q32.1 **BP** [78]	YES	Increased in subjects with **suicide** as cause of death [79]	4/6
AI841629	GNAI2 - guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	**NA** 1.15/ 1.32	YES		3p21 **SZ** [5]	YES	Decreased in PFC **suicide** [81] [105]	4/6
AB006361	PTGDS - Prostaglandin D synthetase	**AMY** 1.41/ 1.32	YES		9q34.3 **SZ** [28]	YES	Decreased in **AD** [106]	4/6
M19279	GUSB - glucuronidase, beta	**CP** 1.23/ 1.32	YES		7q11.21	YES	**MPS VII** [107]	3/6
U40930	SQSTM1 - Sequestosome 1 ubiquitin-binding protein p62	**AMY** 1.23/ 1.32	YES		5q35.3	YES	Early accumulation in neurofibrillary tangles in **AD** [108]	3/6
AI844797	SCN4B - sodium channel, voltage-gated, type IV, beta	**PFC** 1.52/ 1.62	YES		11q23.3 **SZ** [5]	YES		3/6
X04017	SPARC - secreted protein, acidic, cysteine-rich (osteonectin	**NA** 1.74/ 1.41	YES		5q33.1 **BP, SZ** [109]	YES		3/6
X81580	IGFBP2 - insulin-like growth factor binding protein 2	**CP** 2.14/ 1.23	YES		2q35 **MDD** [110]	YES		3/6
L07264	DTR (HB-EGF) - diphtheria toxin receptor	**CP** 1.32/ 1.52	YES		5q31.2 **SZ** [111]	YES		3/6
Down								
L25274	ALCAM - activated leukocyte cell adhesion molecule	**PFC** METH 0.76/ 0.71	YES	**AMY** VPA IV 0.66/ 0.5 **VT** METH IV 0.76/ 0.62	3q13.1 **SZ** [34]	YES		4/6
AI852174	CHN1 - Chimerin 1	**NA** METH 0.58/ 0.76	YES	**AMY** VPA IV 0.81/ 0.33 **CP** VPA IV 1.41/ 4.29	2q31.1 **BP** [112]	YES		4/6
AW050231	MAPT - microtubule-associated protein tau	**AMY** 0.76/ 0.71	YES		17q21.31 **BP** [4]	YES	**BP** [113], dementia [114]	4/6
AW050323	SYNPO - synaptopodin	**PFC** 0.76/ 0.81	YES		5q33.1 **BP, SZ** [109]	YES		3/6
	Valproate Changed							
Up								
AB005664	JNK2 (MAPK9) - c-Jun N-terminal kinase 2	**CP** 1.52/ 1.74	YES	**AMY** IV 0.81/ 0.5	5q35	YES	Activation in **AD** [115]	4/6
X95818	SYP - synaptophysin	**AMY** 1.41/ 1.52	YES		xp11.23-p11.22 **SZ** [116]	YES	Decreased in hippocampus of **SZ** and BP [22]. Decreased in gyrus cinguli of **SZ** [117]	4/6
AW125370	NCS-1 (FREQ) - neuronal calcium sensor	**AMY** 1.23/ 1.52	YES		9q34.11 **SZ** [28]	YES	Increased in PFC of **SZ** and **BP** [118]	4/6
U58513	ROCK-2 - Rho-associated, coiled-coil containing protein kinase 2	**PFC** 1.41/ 1.32	YES	**AMY** IV 0.81/ 0.62	2p24	YES		3/6
M35725	SOD1 - Cu-Zn superoxide dismutase	**AMY** 1.32/ 1.87	YES		21q22.11	YES	**ALS** [119]	3/6
M55669	PCSK2 - prohormone convertase 2	**AMY** 1.32/ 1.62	YES		20p12.1 **BP** [78]	YES		3/6
Down								
M16472	PLP1 - proteolipid protein (myelin)	**AMY** 0.81/ 0.29	YES	**CP** IV 1.32/ 4.59	Xq22.2	YES	Decreased in the PFC of **SZ** and **BP** [76]	4/6
AI846289	CSNK1D - casein kinase 1, delta	**AMY** 0.76/ 0.66	YES	**CP** IV 1.52/ 1.52	17q25.3	YES	Increased in **AD** [120]	4/6
U60150	VAMP2 synaptobrevin - vesicle-associated membrane protein 2	**AMY** 0.71/ 0.5	YES	**CP** IV 1.74/ 6.06	17p13.1	YES	Increased in **HD** [121]	4/6
X61455	NAPB (beta-SNAP) - N-ethylmaleimide-sensitive factor attachment protein, beta	**AMY** 0.76/ 0.5	YES		20p11.21 **SZ** [5]	YES	Decreased in **AD** and **DS** [122]	4/6
AV004774	GRM3 - glutamate receptor, metabotropic 3	**PFC** 0.71/ 0.47	YES		7q21.12 **SZ** [26]	YES	Decreased in **SZ** [123]	4/6
AI788757	CCR4 (NOC) - chemokine (C-C motif) receptor 4 nocturnin	**AMY** 0.41/ 0.71	YES		3p24 **SZ** [5]	YES		3/6
AB003433	CRY2 - cryptochrome 2	**AMY** 0.66/ 0.62	YES	**CP** IV 1.74/ 2	11p11.2	YES		3/6
AI853311	NDRG4 - N-myc downstream regulated 4	**AMY** 0.76/ 0.44	YES	**CP** IV 1.51/ 3.73	16q21	YES		3/6
AW122015	SPIN - spindlin	**AMY** 0.62/ 0.44	YES	**CP** IV 1.41/ 1.62	9q22.1 **BP** [4]			3/6
AA637320	IDS - iduronate 2-sulfatase	**AMY** 0.71/ 0.38	YES	**CP** IV 1.52/ 2.46	Xq28		Absent in Hunter's syndrome [124]	3/6
AF071313	COPS3 - COP9 (constitutive photomorphogenic) homolog, subunit 3 (Arabidopsis thaliana)	**CP** 0.76/ 0.66	YES	**AMY** IV 1.23/ 1.15	17p11.2 **BP** [97]			3/6
AF053473	KIF5A - kinesin family member 5A	**AMY** 0.76/ 0.35	YES	**CP** IV 1.62/ 3.03	12q13.3	YES		3/6
U13836	ATP6V0A1 - ATPase, H+ transporting, lysosomal V0 subunit a isoform 1	**AMY** 0.76/ 0.47	YES	**CP** IV 1.41/ 3.25	17q21 **BP** [4]			3/6
AV231065	KIAA1363	**AMY** 0.66/ 0.10	YES	**CP** IV 1.23/ 4.29	3q26.31 **BP** [75]			3/6
AW122655	HIS1 - cardiac lineage protein 1	**AMY** 0.76/ 0.71	YES	**CP** IV 1.52/ 2.83	17q21.31 **BP** [4]			3/6
AI838022	ARF3 - ADP-ribosylation factor 3	**AMY** 0.76/ 0.57	YES	**CP** IV 1.41/ 2.30	12q13.12			3/6
AI507519	DAPK1 - death-associated protein kinase 1	**AMY** 0.87/ 0.81	YES		9q21.33 **SZ** [125]	YES		3/6
AW048257	PDE2A - Phosphodiesterase 2A	**AMY** 0.62/ 0.66	YES	**CP** IV 1.32/ 4.92	11q13.3	YES		3/6
L20343	CACNB2 - calcium channel, voltage-dependent, beta 2 subunit	**AMY** 0.66/ 0.54	YES	**CP** IV 1.15/ 2.46	10p12.33	YES		3/6
M14220	NLK - neuroleukin	**CP** 0.71/ 0.57	YES		19q13.1	YES	**HD** [126]	3/6

Category III genes with a minimum of three out of six lines of evidence are shown. *Fold changes and *P*-values were calculated using the Affymetrix MASv5.0 analysis software. All *P*-values were ≤ 0.0024. Up: upregulated; Down: downregulated; Meth: methamphetamine; VPA: valproate; PFC: prefrontal cortex; AMY: amygdala; CP: caudate putamen; NA: nucleus accumbens; VT: ventral tegmentum; BP: bipolar disorder; SZ: schizophrenia; MDD: major depressive disorder; AD: Alzheimer. Roman numerals in the multiple brain region data column represent the category of the gene.
MPS VII: mucopolysaccharidosis VII; ALS: amyotrophic lateral sclerosis; HD: Huntington's disease; DS: Down's syndrome.

Table 3 Top category IV genes

Mouse Accession Number	Symbol - Description	Brain region Fold Change	Stopped by Co-Treatment	Multiple Brain Region	Convergent Functional Genomics	Biology	Human postmortem	No. of lines of evidence
	Methamphetamine Changed							
Up								
X02801	GFAP - glial fibrillary acidic protein	CP 4/ 2.83		NA 1.62/ 1.41	17q21.31 BP [4]	YES	Decreased levels in frontal cortex of SZ [127], MDD [128]	4/6
M31811	MAG - myelin associated glycoprotein	CP 1.41/ 1.32			19q13.12 RDP [38]	YES	Decreased in SZ [129]	3/6
AJ238309	DAT1 - SLC6A3 solute carrier family 6 (neurotransmitter transporter, dopamine), member	VT 1.41/ 1.74			5p15.3 BP [130]	YES	Decreased in caudate from THC (-) subjects with SZ [131]. Decreased expression in SZ. [132]	3/6
Down								
X55573	BDNF - brain-derived neurotrophic factor	PFC 0.76/ 0.71			11p14.1 BP [64 65]	YES	Decreased in SI [67]. Increased in subjects treated with antidepressant medications at time of death [133]. Increased in hippocampus of SZ [134]. Decreased in MDD and BP [66]	3/6
X57497	GRIA1 - glutamate receptor, ionotropic, AMPA 1	VT 0.76/ 0.66			5q33.2 SZ [5]	YES	Decrease in binding in SZ [135]	3/6
	Valproate Changed							
Down / Up								
AF058799	14-3-3 gamma (YWHAG) - 3-monooxgenase /tryptophan 5-monooxgenase actation protein, gamma polypeptide	AMY 0.43/ 0.31		CP 1.87/ 24.3 VT 1.32/ 1.41	7q11.23 SZ [136]	YES	Associated with Parkinson disease and diffuse Lewy body disease [137].	4/6
AI842094	CGEF2 - cAMP-regulated guanine nucleotide exchange factor II	AMY 0.54/ 0.54		CP 1.52/ 2.64	2q31.1 BP [112]	YES		3/6
Up								
Z23077	AMD1 - S-adenosylmethionine decarboxylase 1	PFC 1.62/ 1.62		CP 1.52/ 2.14	6q21 BP [37]	YES		3/6
J04192	CHRM1 - cholinergic receptor, muscarinic 1	CP 1.52/ 3.03			11q12.3 SZ [5]	YES	Decreased in CP of SZ [138]	3/6
AI847120	GRIN1 (NMDA-1) - glutamate receptor, ionotropic, N-methyl D-aspartate 1	CP 2.14/ 12.13			9q34.3 BP [139]	YES	Increased in PFC of SZ [140]	3/6
AB004315	RGS4 - regulator of G-protein signalling 4	CP 1.52/ 10.56			1q23.3 SZ [141]	YES	Decreased in PFC of SZ [21]	3/6
AF100956	DAXX - death-associated protein 6	AMY 1.32/ 1.32			6p21.3, SZ [142]	YES	Increased in hippocampus of AD [143]	3/6
M21041	MAP2 - microtubule-associated protein 2	CP 1.87/ 34.3			2q34-q35 MDD [110]	YES	Decreased in BP [144]	3/6
	Methamphetamine / Valproate Changed							
Down / Up								
D10011	KA2 (GRIK5) - glutamate receptor, ionotropic, kainate 5	CP METH 0.81/ 0.66		AMY VPA 1.32/ 1.62	19q13.2 RDP [38]	YES	Decreased in PFC of SZ [145]	4/6
AI841733	PCSK1N - proprotein convertase subtilisin/kexin type 1 inhibitor	CP METH 0.81/ 0.66		AMY VPA 1.41/ 1.52	xp11.23 BP [116]	YES	Pick's disease [146]	4/6
Down								
AW121087	GSK3B - Glycogen synthase kinase 3 beta	PFC METH 0.54/ 0.62		CP VPA 0.76/ 0.71	3q13.3 SZ [34]	YES	Reduced activity in AD [147]. Decreased in SZ [148]	4/6

Category IV genes with a minimum of three out of six lines of evidence are shown. *Fold changes and *P*-values were calculated using the Affymetrix MASv5.0 software. All *P*-values were ≤ 0.0029. Up: upregulated; Down: downregulated; Meth: methamphetamine; VPA: valproate; PFC: prefrontal cortex; AMY: amygdala; CP: caudate putamen; NA: nucleus accumbens; VT: ventral tegmentum; BP: bipolar disorder; SZ: schizophrenia; MDD: major depressive disorder; AD: Alzheimer disease; RDP: rapid-onset dystonia-parkinsonism.

Other examples of individual candidate genes in our data set that have been implicated in pain regulation are neuropeptide Y receptor 2[54] (Table 2) and BDNF[55] (Table 3).

Moreover, at the level of groups of genes, our approach identified a series of glutamate and GABA-related genes as candidate genes (Table 5). Evidence from experimental pain research has revealed that metabotropic glutamate receptors (mGluRs) play a pivotal role in nociceptive processing, inflammatory pain, and hyperalgesia.[56] Hyperalgesia during opioid abstinence is mediated by both glutamate and substance P.[57] GABA mechanisms have been implicated in how cerebral cortex activity can change the set-point of pain threshold in a top-down manner.[58]

Taken together, our data support the existence of a genetic and neurobiological overlap between mood, pain, and pleasure pathways.

Infrastructure changes

MEF2C (myocyte enhancer factor 2C) levels are increased by valproate, and decreased by methamphetamine, in the PFC (Table 1). Cotreatment with both drugs prevents changes. MEF2C is a transcription factor that has been implicated in activity-dependent neuronal cell survival[59] and neurogenesis,[60] as well as in the CREB and ΔFosB mediated response to cocaine.[61] Cdk5 inhibits MEF2C activity, removing an impediment to neuronal cell death under neurotoxic conditions,[62] while BDNF

(brain-derived neurotrophic factor), conversely, activates the ERK5-MEF2 signaling pathway as part of its neurotrophic effects.[63] Interestingly, BDNF located at 11p14.1[64,65] is decreased in the PFC by methamphetamine in our data set (Table 3), and is reported decreased in depressed patients and suicide victims.[66,67] Taken together, our data support a central downstream role for MEF2C in mediating the neuronal infrastructure changes associated with bipolar disorder, and is consistent with a neuroprotective role for mood stabilizers.[68]

Another top candidate gene we have identified that may play a role in brain infrastructure is TBR1 (T-box brain gene 1). TBR1 is a putative transcription factor that is highly expressed in glutamatergic early-born cortical neurons. In TBR1-deficient mice, these early-born neurons had molecular and functional defects. Cajal-Retzius cells expressed decreased levels of Reelin, and impaired subplate differentiation was associated with ectopic projection of thalamocortical fibers into the basal telencephalon. Thus, TBR1 is thought to orchestrate cortical development.[69] TBR1 was also reported changed in the CREB and ΔFosB-mediated response to cocaine.[61] Notably, it is increased in human postmortem bipolar disorder brains,[66] and it maps in a linkage loci for schizophrenia (2q24.2).[5] TBR1 was decreased in our data set in the PFC by both methamphetamine and valproate (Table 1), which may underlie the cognitive impact and longer term (side) effects of these drugs, and of bipolar and related disorders proper.

Behavioral correlates of gene expression

We hypothesized *a priori* that genes that would be changed in expression by both methamphetamine and valproate single-drug treatment might show changes in opposite directions, that is, increased in one case, decreased in the other, and *vice versa*. This proved not to be the case for the majority of genes. In retrospect, our hypothesis was simplistic. The behavioral data (Figure 4) are consistent with both the mice on methamphetamine and the mice on valproate having a similar phenotype at 24 h. While their phenotypes were clearly different in the initial assessment at 30 min following injection (Figure 4a, c), showing the activating effects of methamphetamine, by 24 h the behavioral parameters were in the same direction (Figure 4b, d), suggesting that the mice on methamphetamine had entered the withdrawal, depressive side of the bipolar disorder phenomenology mimicked by methamphetamine (Figure 1a). Valproate *per se* has overall an antiactivating, depressant-like effect[70] in the baseline high-activity C57Bl6 strain of mice that was used for our experiments (data not shown). In humans also, valproate, and lithium, provide greater benefits for prevention of manic relapses and control of manic symptomatology than for depression. Several studies indicate actual worsening in depressive aspects of bipolar disorder with mood stabilizer treatment.[14] In line with the above, in our data set, the vectors of change in some of the known genes, such as BDNF, which is decreased (Table 3), and TAC1 (substance P) and PENK (preproenkephalin), which are both increased (Table 1), are consistent with their reported changes in depression-line paradigms.[71] Nevertheless, more extensive time courses and gene expression–behavioral correlation work needs to be carried out, in both groups of animals[72] and individual animals, in order for a complete picture to emerge linking different behavioral parameters with changes in specific genes or groups of genes.

Gene Ontology (GO) analysis results

GO analysis of the complete data set, categories I–IV (Table 4), revealed that the highest probability genes were genes having to do with (1) cell communication, (2) infrastructure (cell growth and/or maintenance, metabolism, morphogenesis), (3) response to stress and other external stimuli, and (4) behavior. This is consistent with a model of bipolar and related disorders that might be speculated to involve a reaction to external stimuli in the form of modified cell communication, infrastructure changes/tissue remodeling, and a consequent altered behavioral output (Figure 5b). Of note, the functional groups conserved across multiple brain regions had to do with cell communication and infrastructure, whereas the environmental input and behavioral output genes were limited to just one or another brain region.

Our approach described thus far is to generate data in an appropriate discovery paradigm, and let the data coalesce into possible mechanistic interpretations. An opposite, hypothesis-driven approach for mining our data set is to interrogate if genes related to known biological mechanisms of interest (Table 5), linkage loci (Table 6), or postmortem findings (Table 7) are present in it—spanning the spectrum from the more sensitive (biological) to the more specific (postmortem) external corroborative lines of evidence.

Biological roles

An interrogation of our complete data set of reproducibly changed genes, categories I–IV, for classification in functional groups that had been previously implicated or hypothesized to have relevance to the pathophysiology of bipolar and related disorders yielded genes related to neurotransmitters (GABA, glutamate, serotonin, dopamine, acetylcholine, adenosine, glycine, neuropeptides), cellular mechanisms (DARPP-32 pathway, clock genes, G-protein-coupled receptor-related genes, inositol pathway, *S*-adenosyl methionine (SAMe)-related genes, transporters), cell compartments (synaptic, Golgi/ER), and physiological functions (pain pathway genes, inflammatory pathway genes, cell survival and death, oxidative stress, and glia-related genes) (Table 5).

Circadian rhythms

Clock genes are especially intriguing candidate genes for bipolar disorder, due to the cyclical nature of the

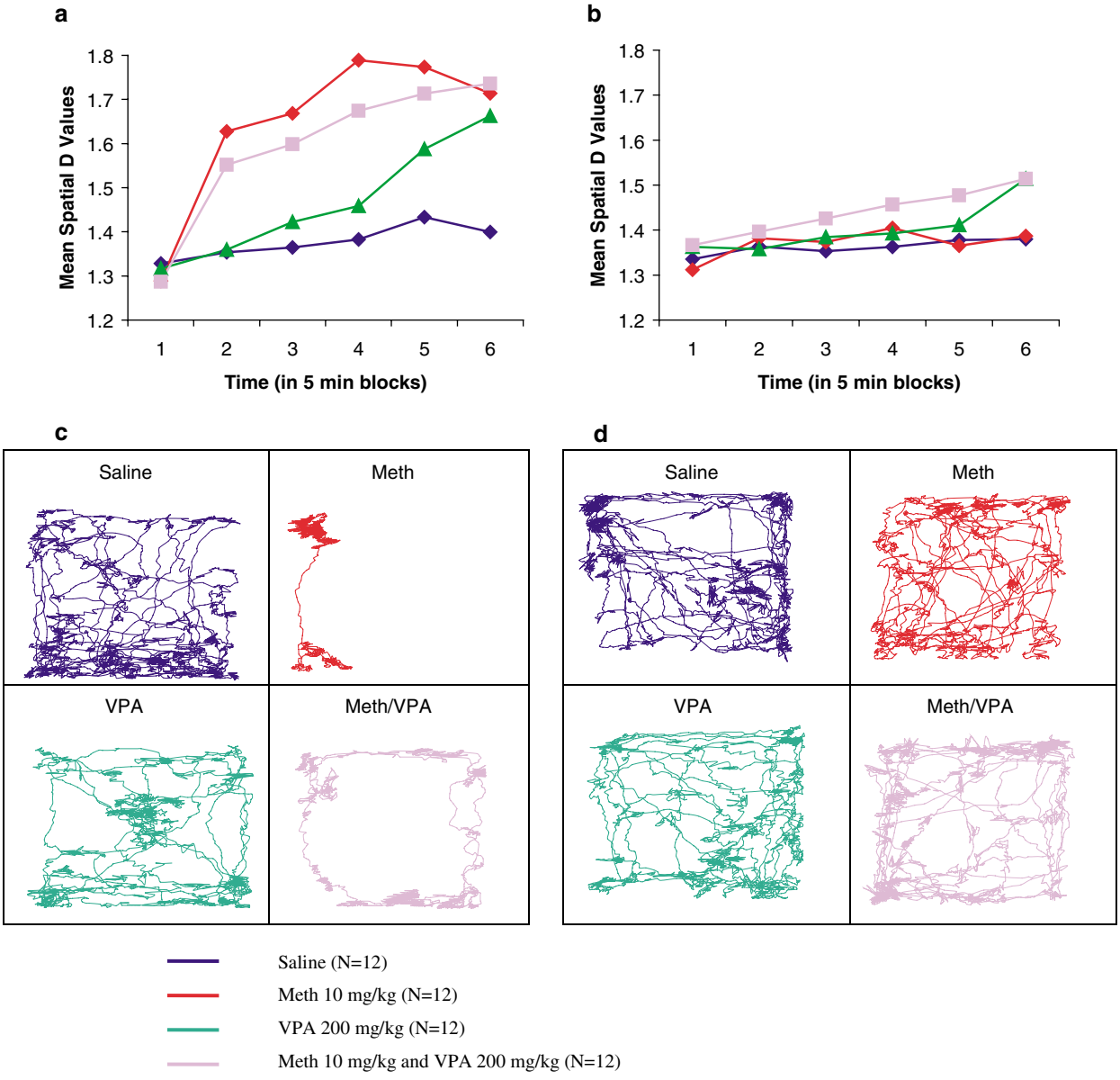

Figure 4 Behavioral correlates of methamphetamine and valproate treatment. (a, b) Behavioral organization and stereotypal behavior. Mean spatial *D* values in first 30 min after injection (a), and repeat measure 24 h after injection (b). Representative individual mice movement patterns in a 5 min (between 15 and 20 min) interval after injection (c), and a repeat measure 24 h later (d). Patterns from representative animals, with spatial *D* values closest to the mean for its group during the 15–20 min time intervals, are illustrated.

illness. Our data set contains three, Category III, such genes (cryptochrome 2 located at 11p11.2, CCR4/nocturnin located at 3p24,[5] and casein kinase I delta located at 17q25.3), which are changed in response to valproate and the change is prevented by methamphetamine cotreatment, and one Category IV gene (BMAL1/MOP3 located at 11p15.2) that is changed in response to methamphetamine (Table 5).

Myelin-related genes

We have a series of myelin-related genes that show changes in our data sets (Table 5). Earlier work has identified oligodendrocyte pathology, and myelin-related genes as candidate genes for schizophrenia.[73,74] More recently, PLP1 (proteolipid protein 1) (Table 2) located at Xq22.2 and CLDN11 (claudin 11) (Table 1) located at 3q26.2,[75] two of our top candidate genes, were shown to be down-regulated in postmortem brains from both schizophrenic and bipolar disorder patients.[76] Taken together, our data support a possibly direct, early role for myelin-related genes and glia in the pathophysiology of bipolar and related disorders, rather than it being secondary, age-related or a postmortem artefact.

Table 4 GO analysis

a

		CATEGORIES					
		I	II	III METH	III VPA	IV METH	IV VPA
GO ANALYSIS – BIOLOGICAL PROCESSES –		NUMBER OF GENES					
1.	Cell communication	2	4	9	12	18	36
2.	Cell growth and/or maintenance	1	5	10	20	25	56
3.	Metabolism	2	9	12	19	35	72
4.	Morphogenesis	1	4	2	4	6	14
5.	Response to stress	1		2	1	5	1
6.	Response to external stimuli	1		2	2	7	2
7.	Reproductive behavior	1				1	
8.	Behavioral fear response	1					
9.	Cell motility		1	2	3	1	3
10.	Homeostasis		1	1			3
11.	Reproduction		1		1		2
12.	Pattern specification		1			1	1
13.	Embryonic development		1				
14.	Cell differentiation			2	2		3
15.	Learning and/or memory				1	1	2
16.	Cell death				1	1	2
17.	Death				1	1	2
18.	Circulation			4			1
19.	Rhythmic behavior				1		1
20.	Genetic transfer				1		
21.	Bone remodelling					1	1
22.	Hemostasis					1	
23.	Regulation of gene expression epigenetic					1	
24.	Membrane fusion						1
25.	Secretion						2

b

Drug / Brain regions		METH			VPA				
		PFC/CP	PFC/VT	CP/NA	AMY/CP/VT	PFC/AMY	AMY/CP	AMY/VT	CP/VT
GO ANALYSIS – BIOLOGICAL PROCESSES		NUMBER OF GENES							
1.	Cell communication		1		1	1	7	1	1
2.	Cell growth and/or maintenance	1			1		12	1	1
3.	Metabolism		1	1	1	1	10	1	1
4.	Morphogenesis						1		
14.	Cell differentiation						1		
19.	Rhythmic behavior						1		
25.	Secretion						1		

Biological processes obtained from the GO analysis: (a) analysis of our complete data set, (b) analysis of genes found in multiple brain regions. Categories I–IV. Meth: methamphetamine; VPA: valproate; PFC: prefrontal cortex; AMY: amygdala; CP: caudate putamen; NA: nucleus accumbens; VT: ventral tegmentum.

Suicidality

Bipolar disorder and related disorders have also been associated clinically with an increased risk of suicide.[77] Six of the candidate genes in our complete data set—NPY2R (neuropeptide Y receptor 2) located at 4q32.1,[78] 5HTR2C (serotonin receptor 2C) located at Xq23, CCK, BDNF, GNAI2 (G protein alpha-inhibiting activity polypeptide 2) located at 3p21,[5] and PTEN (phosphatase and tensin homologue) (Tables 5 and 7) located at 10q23.3, have been implicated in both animal models of mood disorders and in postmortem studies of suicide victims.[67,79–83] It is interesting to note that at least five of them, NPY2R, 5HTR2C, CCK, BDNF, and PTEN, modulate food intake and related metabolic functions, and are thought to be involved in obesity. It may thus be of some interest to explore in future studies the epidemiological correlations between body weight and suicidality. Taken together, our data are consistent with the possibility that a strong negative correlation may exist, with excessive food intake acting as a suicide-mitigating factor.

Crossvalidation with human linkage loci

Interrogating our data set for genes that map to the linkage loci reported by recent meta-analyses for bipolar disorder and schizophrenia yielded a series of candidate genes at those loci (Table 6) that may help prioritize future candidate gene research for each

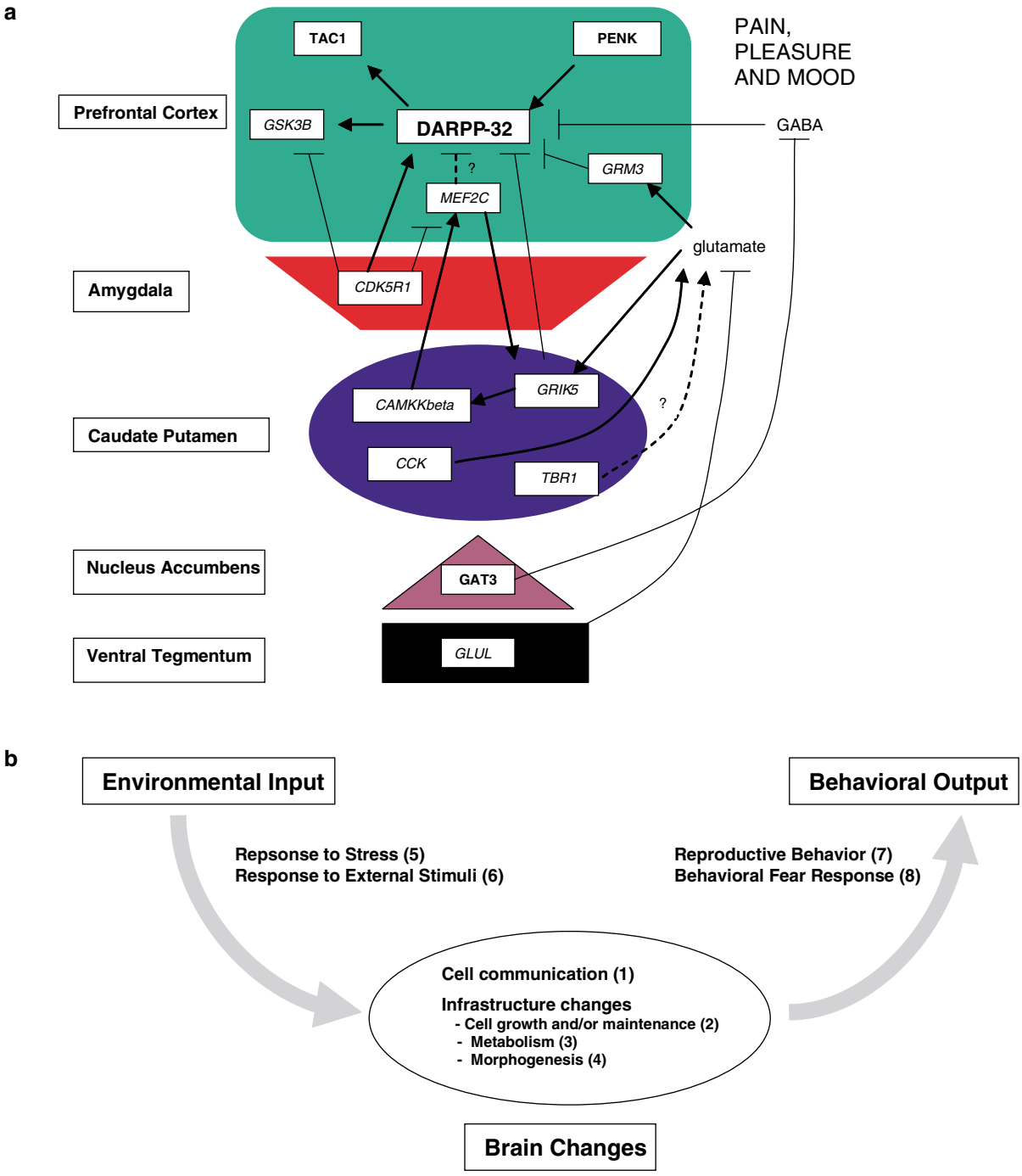

Figure 5 Candidate genes, pathways and mechanisms. (a) Top candidate genes and their relationships to DARPP-32. All the Category I genes, all the genes with five out of six lines of evidence, and all the genes with four out of six lines of evidence that have a known relationship with DARPP-32 are illustrated. Genes are depicted in the brain region in which they were reproducibly changed. For genes that showed changes in multiple brain regions, the gene is depicted in the brain region in which that gene showed the most lines of evidence. Genes in plain text are increased by methamphetamine, and genes in italics are decreased by methamphetamine. Broken lines indicate the relationship is still hypothetical. (b) GO analysis-derived model of biological processes and mechanisms. Numbered categories refer to GO analysis categories from Table 4.

of the loci. It is to be noted that the meta-analyses did not reproduce all the previous linkage results,[2,3] some of which were used by us to determine convergence to a linkage loci for our top candidate genes (Tables 1–3). While this remains an open question, it may be due to the fact that meta-analyses may replicate loci having to do with general genes that are involved in all of the multiple forms of bipolar disorder, across different

Table 5 Candidate genes and biological roles

Mouse Accession Numbers	Gene / Name	Brain Region (Drug-Category)
NEUROTRANSMITTERS		
GABA		
	Up	
AW120565	SLC6A11 (GAT3) solute carrier family 6 member 1	NA (II)
U14420	GABRB3 gamma-aminobutyric acid (GABA) A receptor, beta 3	CP (VPA-IV)
M62374	GABRG2 gamma-aminobutyric acid (GABA) A receptor, gamma2	CP (VPA-IV)
AI606317	GABRA4 gamma-aminobutyric acid (GABA) A receptor, alpha 4	PFC (METH-IV)
Glutamate		
	Up / Down	
D10011	GRIK5 glutamate receptor, ionotropic, kainate 5	AMY(VPA-IV) / CP (METH-IV)
	Up	
AI847120	GRIN1 glutamate receptor, ionotropic, N-methyl D-aspartate 1	CP (IV-VPA)
	Down	
AI848384	GLUL glutamate-ammonia ligase (glutamine synthase)	VT (II) PFC (METH-III)
X57497	GRIA1 glutamate receptor, ionotropic, AMPA 1	VT (METH-IV)
AV004774	GRM3 glutamate receptor, metabotropic 3	PFC (VPA-III)
Serotonin		
	Up	
M63685	5HTR2C 5-hydroxytryptamine (serotonin) receptor 2C	CP (METH-IV)
Dopamine		
	Up	
AJ238309	DAT (SLC6A3) solute carrier family 6 (dopamine transporter)	VT (METH-IV)
Cholinergic		
	Up	
J04192	CHRM1 cholinergic receptor, muscarinic 1	CP (VPA-IV)
Adenosine		
	Down	
Y13344	ADORA2A adenosine A2a receptor	NA (METH-IV)
Glycine		
	Up	
X81202	GLRB glycine receptor, beta	CP (METH-IV)
Neuropeptide		
	Up	
D17584	TAC1 Tachykinin 1 - substance P - tachykinin, precursor 1	PFC (I)
AV372577	NPY2R neuropeptide Y receptor Y2	NA (METH-III)
AI322575	CART cocaine- and amphetamine-regulated transcript	VT (METH-IV)
	Down	
X59520	CCK Cholecystokinin	CP (II) NA (METH-IV)
AB010149	ADCYAP1 adenylate cyclase activating polypeptide 1 (pituitary)	VT (VPA-IV)
CELLULAR MECHANISMS		
DARPP-32 pathway		
	Up	
AI839758	DARPP-32- dopamine- and cAMP- regulated phosphoprotein of 32 kilodaltons	PFC (I)
AI850402	PPP1R16B protein phosphatase 1, regulatory (inhibitor) subunit 16B	AMY (VPA-IV)
M27073	PPP1CB protein phosphatase 1, catalytic subunit, beta isoform	CP (METH-IV)
AA764532	PPP2R5A protein phosphatase 2, regulatory subunit B, alpha isoform	CP (VPA-IV)
	Down	
AI430766	PPP1R12A protein phosphatase 1, regulatory (inhibitor) subunit 12A	AMY (VPA-IV) CP (VPA-IV)
Circadian clock genes		
	Up / Down	
AI846289	CSNK1D casein kinase 1, delta	CP (VPA-IV) / AMY (VPA-III)
AB003433	CRY2 -Cryptochrome 2	CP (VPA-IV) / AMY (VPA-III)
	Down	
AI837830	MOP3-BMAL1 - Brain and muscle ARNT-like 1	PFC (METH-IV)
AI788757	CCR4 - nocturnin – chemokine (C-C motif) receptor 4	AMY (VPA-III)
G-protein coupled receptors related genes		
	Up / Down	
AI845935	GNB1 guanine nucleotide binding protein (G protein), beta polypeptide 1	AMY (VPA-IV) / CP (VPA-IV)
	Up	
AI852526	GPR88 G-protein coupled receptor 88	PFC (I)
AI841629	GNAI2 Guanine nucleotide-binding protein G(I), alpha-2 subunit	NA (METH-III)
AB004315	RGS4 regulator of G-protein signalling 4	CP (VPA-IV)
AI850107	GNG7 guanine nucleotide binding protein (G protein), gamma 7 subunit	PFC (METH-III)
Inositol pathway		
	Up	
AI747899	PITPNB phosphatidylinositol transfer protein, beta	CP (II)
U32329	EDNRB endothelin receptor type B	CP (METH-III)
M21530	ITPR1 Inositol 1,4,5-triphosphate receptor, type 1	CP (VPA-IV)
AB009615	PIP5K2A phosphatidylinositol-4-phosphate 5-kinase, type II, alpha	VT (VPA-IV)
	Down	
AW124994	SYNJ1 synaptojanin 1 Inositol 5-phosphatase	AMY (VPA-IV)
AW123736	PIP5K1C phosphatidylinositol-4-phosphate 5-kinase, type I, gamma	CP (METH-IV)
PKC pathway		
	Up / Down	
AF058799	14-3-3 YWHAG - tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	CP (VPA-IV), VT (VPA-IV) / AMY (VPA-IV)
Phosphatidic acid pathway		
	Up	
U89352	LYPLA1 Lysophospholipase I	CP (I)

Mouse Accession Numbers	Gene / Name	Brain Region (Drug-Category)
CELLULAR MECHANISMS (CONT.)		
S-adenosyl methionine (SAMe) related genes		
	Up	
AI837110	HRMT1L2 hnRNP methyltransferase-like 2, PRMT1	NA (II)
Z23077	AMD1 S-adenosylmethionine decarboxylase 1	PFC (VPA-IV) CP (VPA-IV)
Transporters		
	Up	
AA082013	SLC25A5 solute carrier family 25 (adenine nucleotide translocator), member 5	CP (VPA-IV)
M75135	GLUT3 SLC2A3 solute carrier family 2 (facilitated glucose transporter), member 3	CP (VPA-IV)
	Down	
AF020195	SLC4A4 Pancreas sodium bicarbonate cotransporter	AMY(VPA-IV) CP(VPA IV)
CELL COMPARTMENTS		
Synaptic		
	Up / Down	
D37792	SYT1 Synaptotagmin 1	CP (II), VT (VPA-IV) / AMY (VPA-IV)
U60150	VAMP2 Synaptobrevin	CP (VPA-IV) / AMY (VPA-III)
	Up	
X95818	SYP Synaptophysin	AMY (VPA-III)
	Down	
AW122328	NPTX1 neuronal pentraxin 1	CP (II)
Golgi/ER		
	Up / Down	
AI838022	ARF3 ADP-ribosylation factor 3	CP(VPA-IV) /AMY(VPA-III)
AI841733	PCSK1N proprotein convertase subtilisin/kexin type 1 inhibitor	AMY(VPA-IV) / CP(METH IV)
	Up	
AI849207	GORASP2 Golgi reassembly stacking protein 2	AMY (II)
AB015426	FUT9 fucosyltransferase 9	CP (METH-IV)
AW227412	GOLPH3 golgi phosphoprotein 3 (coat-protein)	CP (VPA-IV)
AI154073	SNX1 sorting nexin 1	CP (VPA-IV)
AI847496	SNX5 sorting nexin 5	CP (VPA-IV)
	Down	
X80502	SIAT8C sialyltransferase 8C	VT (METH-IV)
X84037	MG-160 GLG1 golgi apparatus protein 1	VT (VPA-III)
AI836688	FBXW7 F-box and WD-40 domain protein 7	AMY (VPA-IV)
AI450216	NAPG N-ethylmaleimide attachment protein gamma	AMY (VPA-IV)
PHYSIOLOGICAL FUNCTIONS		
Pain pathways		
	Up	
D17584	TAC1 Tachykinin 1 - substance P - tachykinin, precursor 1	PFC (I)
M55181	PENK Preproenkephalin 2	PFC (I)
Inflammatory pathways		
	Up/Down	
AB005664	JNK2 (MAPK9) - c-Jun N-terminal kinase 2	CP(VPA-III)/ AMY(VPA-IV)
Cell survival / death		
	Up / Down	
AF058799	14-3-3 YWHAG - tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	CP (VPA-IV), VT (VPA-IV) / AMY (VPA-IV)
U58513	ROCK-2 Rho-associated, coiled-coil containing protein kinase 2	PFC(VPA-III)/AMY(VPA -IV)
	Up	
X81580	IGFBP2 insulin-like growth factor binding protein 2	CP (METH-III)
X66449	S100A6 S100 calcium binding protein A6 (calcyclin)	CP (METH-III)
D90225	PTN pleiotrophin	CP (METH-IV)
M20473	PRKAR1b protein kinase, cAMP-dependent, regulatory, type I, beta	CP (VPA-IV)
AI314322	PRKAR2b protein kinase, cAMP-dependent, regulatory, type II, beta	CP (VPA-IV)
	Down	
AW121087	GSK3B Glycogen synthase kinase 3 beta	PFC (METH-IV) CP (VPA-IV)
AI507519	DAPK1 death-associated protein kinase 1	AMY (VPA-III)
X55573	BDNF brain-derived neurotrophic factor	PFC (METH-IV)
AV102186	BAD BCL2-antagonist of cell death	AMY (VPA-IV)
AI854469	SON SON DNA binding protein	AMY (VPA-III)
Oxidative stress		
	Up	
M35525	SOD1 Cu-Zn superoxide dismutase	AMY (VPA-III)
Glia / Myelin		
	Down / Up	
M16472	PLP1 Mouse proteolipid protein variant DM-20 mRNA	AMY (VPA-III) / CP (VPA-IV)
	Up	
U81317	MOBP myelin-associated oligodendrocytic basic protein	CP (II) VT (VPA-IV)
X02801	GFAP glial fibrillary acidic protein	CP (METH-IV) NA (METH-IV)
U19582	CLDN11 Oligodendrocyte specific protein	CP (II)
U48836	UGT8 UDP-glucuronosyltransferase 8	CP (II)
Z38110	PMP22 peripheral myelin protein 22	CP (METH-IV)
M31811	MAG myelin associated glycoprotein	CP (METH-IV)
	Down	
X16645	AMOG ATPase, Na+/K+ transporting, beta 2 polypeptide	AMY (VPA-IV)
AI159117	GMFB glia maturation factor, beta	AMY (VPA-IV)

Additional Evidence: ■ Linkage ■ Postmortem

Genes from our complete data set were classified into biological groups of interest previously reported to have relevance to the pathophysiology of bipolar and related disorders. Blue dots indicate whether or not the gene also maps to a linkage locus associated with bipolar disorder, schizophrenia, or depression. Green dots indicate whether or not there are also data showing human postmortem alterations in expression of that gene in brains from patients with bipolar disorder, schizophrenia, depression, or other brain disorders that impact mood and cognition. Up: upregulated; Down: downregulated; Meth: methamphetamine; VPA: valproate; PFC: prefrontal cortex; AMY: amygdala; CP: caudate putamen; NA: nucleus accumbens; VT: ventral tegmentum. Roman numerals in the brain region data column represent the category of the gene.

Table 6 Candidate genes mapping to meta-analyses linkage loci

Bipolar

Loci	Symbol	Description
1q32.3		
1q32.1	PIGR	polymeric immunoglobulin receptor
1q32.3	PPP2R5A	protein phosphatase 2, regulatory subunit B (B56), alpha isoform
2q23.3		
2q24.2	TBR1	T-box brain gene 1
2q25.31	PKP4	plakophilin 4
2q23.3	REPRIMO	candidate mediator of the p53-dependent G2 arrest
3q25.33		
3q26.2	CLDN11	Oligodendrocyte specific protein
3q25.31	SSR3	signal sequence receptor, gamma
3q25.1	TAZ	transcriptional co-activator with PDZ-binding motif
3q25.1	RNF13	ring finger protein 13
3q25.2	RAP2B	Ras-related protein RAP-2b
3q25.31	KCNAB1	potassium voltage-gated channel, shaker-related subfamily, beta member
5p15.1		
5p15.1-p14	BASP1	brain abundant, membrane attached signal protein 1
8p22		
8p22	CNOT7	CCR4-associated factor 1
8p23.1	MTMR9	myotubularin related protein 9
8p23.1	LPAAT-e	acid acyltransferase-epsilon
9p21.1*		
9p21.3	ELAVL2	embryonic lethal, abnormal vision, Drosophila-like 2
9q21.32		
9q21.32	UBQLN1	ubiquilin 1
9q22.1	SPIN	spindlin
9qter		
9q34.3	PTGDS	Prostaglandin D synthetase
9q34.3	OLFM1	olfactomedin 1
9q34.3	GRIN1	glutamate receptor, ionotropic, N-methyl D-aspartate 1
10q22.1*		
10q22.2	CAMK2G	calcium/calmodulin-dependent protein kinase II gamma
10q22.1	PSAP	prosaposin
10q22.1	SGPL1	sphingosine phosphate lyase 1
11q13.4		
11q13.4	PDE2A	phosphodiesterase 2A
11q13.1	BAD	BCL2-antagonist of cell death
11q13.5	SERPINH1	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1
11q13.5	PAK1	p21/Cdc42/Rac1-activated kinase 1
14q32.12*		
14q32.1	SERPINA3	serine (or cysteine) proteinase inhibitor, clade A , member 3
14q32	DDX24	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 24
14q24.1		
14q24.3	TMP21	transmembrane trafficking protein
17q21.31		
17q12	DARPP-32	dopamine- and cAMP- regulated phosphoprotein of 32 kilodaltons
17q21.31	MAPT	microtubule-associated protein tau
17q21.2	ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit a isoform 1
17q21.31	HIS1	cardiac lineage protein 1
17q21.31	RPL27	Ribosomal protein L27
17q21.33	PRO1855	hypothetical protein PRO1855
17q21.31	FMNL	formin-like
17q21.31	GFAP	glial fibrillary acidic protein
18p11.23		
18p11.22	NAPG	N-ethylmaleimide sensitive fusion protein attachment protein gamma
18q12.2		
18q11.2	AQP4	aquaporin 4
18q12.1	TTR	transthyretin (prealbumin, amyloidosis type I)
19qter		
19q13.43	PEG3	paternally expressed gene 3
19q13.42	KIAA1115	
19q13.42	RPL28	ribosomal protein L28
20p12.3		
20p12.2	BTBD3	BTB (POZ) domain containing 3

Schizophrenia

Loci	Symbol	Description
1q23.3		
1q23.3	RGS4	regulator of G-protein signaling 4
1q31.1		
1q25.3	GLUL	glutamate-ammonia ligase (glutamine synthase)
1q25.3	NS1-BP	NS1-binding protein
1q31.2	SSA2	Sjogren syndrome antigen A2
2q23.3		
2q24.2	TBR1	T-box brain gene 1
2q23.3	REPRIMO	candidate mediator of the p53-dependent G2 arrest
2q24.1	PKP4	plakophilin 4
3p22.1*		
3p22-p21.3	CCK	Cholecystokinin
3p21.33	MOBP	myelin-associated oligodendrocytic basic protein
3p22.3	CLASP2	cytoplasmic linker associated protein 2
3p21.3	RBM5	RNA binding motif protein 5
3p21	GNAI2	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2
3p21.2-p21.1	ITIH3	pre-alpha (globulin) inhibitor, H3 polypeptide
5q34*		
5q33.2	GRIA1	glutamate receptor, ionotropic, AMPA 1
5q34	GABRG2	gamma-aminobutyric acid (GABA) A receptor, gamma 2
5q34	KIBRA	KIBRA protein
6p22.3		
6p22.3	CAP2	adenylyl cyclase-associated protein 2
6p23	SCA1	spinocerebellar ataxia 1
11q24.1*		
11q23.3	SCN4B	sodium channel, voltage-gated, type IV, beta
11q24.2	CHEK1	CHK1 checkpoint homolog
11q23.3	DDX6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6
11q23.3	ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12
11q23.3	THY1	Thy-1.2 glycoprotein gene
14q13.1		
14q12	ARHGAP5	Rho GTPase activating protein 5
14q12	HECTD1	E3 ligase for inhibin receptor mRNA
14q12	STRN3	striatin, calmodulin binding protein 3
15q26.1		
15q26.1	IQGAP1	IQ motif containing GTPase activating protein 1
15q25.2	BTBD1	BTB (POZ) domain containing 1
16q12.2		
16q12.1	TRF4-2	topoisomerase-related function protein 4-2
16q13	MT1A	Metallothionein 1
16q12.1	CYLD	cylindromatosis (turban tumor syndrome)
20p11.23		
20p12.1	PCSK2	Proprotein convertase subtilisin/kexin type II
20p11.21	NAPB	N-ethylmaleimide-sensitive factor attachment protein, beta
20p11.23	SNX5	sorting nexin 5
22q12.3		
22q12.1	PITPNB	phosphotidylinositol transfer protein, beta
22q13.2	ROXAN	ubiquitous tetratricopeptide containing protein RoXaN
22q13.1	LGALS1	lectin, galactoside-binding, soluble, 1
22q13.1	NPTXR	neuronal pentraxin receptor
22q12.3	RBM9	RNA binding motif protein 9

Additional Evidence: Postmortem

Genes from our complete data set mapping to linkage loci identified in the most recent meta-analyses of bipolar disorder[4] and schizophrenia[5] under any disease model. *Average ranks with P_{AvgRnk} values <0.01, denoting the strongest linkage signals in the meta-analyses. The rest of the linkages loci have P_{AvgRnk} values <0.05. All genes listed were within at least 10 cM of the marker for the given chromosomal location. Green dots indicate whether or not there are also data showing human postmortem alterations in expression of that gene in brains from patients.

populations, whereas different individual studies may pick up linkages related to less general, and perhaps more specific genes. It should be further noted that there were some susceptibility loci implicated in both bipolar disorder and schizophrenia that did not come up in our study, such as 13q.[84] This may be due to the fact that we are certainly not capturing all the possible candidate genes for bipolar disorder and schizophrenia with the model and approach described in this paper.

Crossvalidation with human postmortem findings

Lastly, an interrogation of our data set with genes that have previously been reported in the literature as

Table 7 Candidate genes and postmortem data

Genes from our dataset (Categories I-IV) with human postmortem changes	Brain region, Drug, Category (Change)
BIPOLAR	
- APOD - apolipoprotein D	**CP** METH IV (U/U)
- BDNF - brain-derived neurotrophic factor	**PFC** METH IV (D/D)
- CLDN11 - Oligodendrocyte specific protein	**CP** II (U/U/U/U)
- FREQ (NCS-1) - frequenin homolog (Drosophila) neuronal calcium sensor	**AMY** VPA III (U/U)
- 5HTR2C - 5-hydroxytryptamine (serotonin) receptor 2C	**CP** METH IV (U/U)
- MAP2 - microtubule-associated protein 2	**CP** VPA IV (U/U)
- MAPT - microtubule-associated protein tau	**AMY** METH III (D/D)
- PITPNB - phosphotidylinositol transfer protein, beta	**CP** II (U/U/U/U)
- PLP1 - proteolipid protein (myelin)	**AMY** VPA III (U/U), **CP** VPA IV (U/U)
- SYP - synaptophysin	**AMY** VPA III (U/U)
- TBR1 - T-box brain gene 1	**CP** II (D/D/D/D), **NA** VPA IV (D/D)
SCHIZOPHRENIA	
- ADORA2A - adenosine A2a receptor	**NA** METH IV (D/D)
- APOD - apolipoprotein D	**CP** METH IV (U/U)
- BDNF - brain-derived neurotrophic factor	**PFC** METH IV (D/D)
- CCK - Cholecystokinin	**CP** II (D/D/D/D), **NA** METH IV (D/D)
- CHRM1 - cholinergic receptor, muscarinic 1	**CP** VPA IV (U/U)
- CLDN11 - Oligodendrocyte specific protein	**CP** II (U/U/U/U)
- CPLX1 - complexin 1	**CP** VPA IV (U/U), **VT** METH IV (U/U)
- DARPP-32 - dopamine- and cAMP- regulated phosphoprotein of 32 kilodaltons	**PFC** I (U/U/U/U)
- DAT1 - SLC6A3 - solute carrier family 6 (neurotransmitter transporter, dopamine), member	**VT** METH IV (U/U)
- FREQ (NCS-1) - frequenin homolog (Drosophila) neuronal calcium sensor	**AMY** VPA III (U/U)
- GFAP - glial fibrillary acidic protein	**CP** METH IV (U/U), **NA** METH IV (U/U)
- GRIA1 - glutamate receptor, ionotropic, AMPA 1	**VT** METH IV (DD)
- GRIK5 (KA2) - glutamate receptor, ionotropic, kainite 5	**AMY** VPA IV (U/U), **CP** METH (D/D)
- GRIN1 (NMDA-1) - glutamate receptor, ionotropic, N-methyl D-aspartate 1	**CP** VPA IV (U/U)
- GRM3 - glutamate receptor, metabotropic 3	**PFC** VPA III (D/D)
- GSK3B - Glycogen synthase kinase 3 beta	**PFC** METH IV (D/D), **CP** VPA IV (D/D)
- 5HTR2C - 5-hydroxytryptamine (serotonin) receptor 2C	**CP** METH IV (U/U)
- LYPLA1 - Lysophospholipase I	**CP** I (U/U/U/U)
- MAG - myelin associated glycoprotein	**CP** METH IV (U/U)
- PENK - Preproenkephalin 2	**PFC** I (U/U/U/U)
- PITPNB - phosphotidylinositol transfer protein, beta	**CP II** (U/U/U/U)
- PLP1 - proteolipid protein (myelin)	**AMY** VPA III (U/U), **CP** VPA IV (U/U)
- RGS4 - regulator of G-protein signalling 4	**CP** VPA IV (U/U)
- SYP - synaptophysin	**AMY** VPA III (U/U)
- SYT1 - Synaptotagmin 1	**CP** II (U/U/U/U), **AMY** VPA IV (D/D), **VT** VPA IV (U/U)
- TAC1 - Tachykinin 1	**PFC** I (U/U/U/U)
DEPRESSION	
- BDNF - brain-derived neurotrophic factor	**PFC** METH IV (D/D)
- GFAP - glial fibrillary acidic protein	**CP** METH IV (U/U), **NA** METH IV (U/U)
- 5HTR2C - 5-hydroxytryptamine (serotonin) receptor 2C	**CP** METH IV (U/U)
- TAC1 - Tachykinin 1	**PFC** I (U/U/U/U)
OTHER	
SUICIDE	
- NPY2R - neuropeptide Y receptor Y2	**NA** METH III (U/U)
- 5HTR2C - 5-hydroxytryptamine (serotonin) receptor 2C	**CP** METH IV (U/U)
- BDNF - brain-derived neurotrophic factor	**PFC** METH IV (D/D)
- CCK - Cholecystokinin	**CP** II (D/D/D/D), **NA** METH IV (D/D)
- GNAI2 - guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	**NA** METH III (U/U)
- PTEN - phosphatase and tensin homolog	**PFC** METH IV (D/D)
OPIATE ADDICTS - CDK5R1-cyclin-dependent kinase 5, regulatory subunit (p35)-	**AMY** II (D/D/D/D), CP VPA III (U/U)
Postmitotic neuronal differentiation in the cortex - MEF2C -MADS box transcription enhancer factor 2	**PFC** I (D/D/U/U), AMY VPA III (U/U)
DOWN SYNDROME	
- SYNJ1 - synaptojanin 1 Inositol 5-phosphatase	**AMY** VPA IV (D/D)
- NAPB (beta-SNAP) - N-ethylmaleimide-sensitive factor attachment protein, beta	**AMY** VPA III (D/D)
ALZHEIMER	
- ADAM10 - a disintegrin and metalloproteinase domain	**CP** II (U/U/U/U)
- APOD - apolipoprotein D	**CP** METH IV (U/U)
- CSNK1D - casein kinase 1, delta	**AMY** VPA III (D/D), **CP** VPA IV (U/U)
- DAXX - death-associated protein 6	**AMY** VPA IV (U/U)
- GLUL - glutamate-ammonia ligase (glutamine synthase)	**VT** II (D/D/D/D)
- GSK3B - Glycogen synthase kinase 3 beta	**PFC** METH IV (D/D)
- JNK2 (MAPK9) - mitogen activated protein kinase 9	**CP** VPA III (U/U), **AMY** VPA IV (D/D)
- LYPLA lysophospholipase	**CP** I (U/U/U/U)
- MAPK10 (JNK3) - mitogen-actated protein kinase 10 - c-Jun N-terminal kinase 3	**CP** VPA IV (D/D)
- MAPT - microtubule-associated protein tau	**AMY** METH III (D/D)
- NAPB (beta-SNAP) - N-ethylmaleimide-sensitive factor attachment protein, beta	**AMY** VPA III (D/D)
- PTGDS - Prostaglandin D synthetase	**AMY** METH III (U/U)
- SQSTM1 - Sequestosome 1 ubiquitin-binding protein p62	**AMY** METH III (U/U)
- WASL - Neural Wiskott-Aldrich syndrome protein (N-WASP).	**CP** VPA IV (U/U)
PARKINSON, LEWY BODY DISEASE - YWHAG (14-3-3 gamma) - 3-monooxgenase /tryptophan 5-monooxgenase activation protein, gamma polypeptide	**AMY** VPA IV (D/D), **CP** VPA IV (U/U), **VT** VPA IV (U/U)
HUNTINGTON'S DISEASE	
- NLK - neuroleukin	**CP** VPA III (D/D)
- SLC2A3 (GLUT3) - solute carrier family 2, member 3	**CP** VPA IV (U/U)
- VAMP2 (synaptobrevin) vesicle-associated membrane protein 2	**AMY** VPA III (D/D), **CP** VPA IV (U/U)
PICK' DISEASE - PCSK1N - proprotein convertase subtilisin/kexin type 1 inhibitor	**AMY** VPA IV (U/U), **CP** METH IV (U/U)
HUNTER'S SYNDROME - IDS - iduronate 2-sulfatase	**CP** VPA IV (U/U)
ALS - SOD1 - Cu-Zn superoxide dismutase	**AMY** VPA IV (U/U)
MPS VII - GUSB - glucuronidase, beta	**CP** METH III (U/U)

Additional Evidence: ■ Linkage

Genes in our complete data set for which there are published reports of alterations in mRNA or protein levels in postmortem brains from patients with bipolar disorder, schizophrenia, depression, or other brain disorders that impact mood and cognition. Blue dots indicate that the gene also maps to a linkage locus associated with bipolar disorder, schizophrenia, or depression. U: upregulated; D: downregulated; Meth: methamphetamine; VPA: valproate; PFC: prefrontal cortex; AMY: amygdala; CP: caudate putamen; NA: nucleus accumbens; VT: ventral tegmentum.

altered in postmortem brains from patients with bipolar disorder, schizophrenia, depression, and other brain disorders that affect mood and cognition, confirmed in our data set some of those earlier findings (Table 7). This crossvalidation, on the one hand, reinforces the validity of our approach and, on the other hand, it reduces the likelihood that those particular postmortem findings are methodological or gene–environment interactions artefacts of working with postmortem human tissue.

Discussion

We have developed an approach for identifying high-probability candidate genes, pathways and mechanisms for complex neuropsychiatric disorders, such as bipolar disorder and related disorders, by the integration in a bayesian pattern of multiple independent converging lines of evidence.

Limitations and confounds

An acute treatment model like the one we are using is not necessarily inductive to assessing the long-term changes associated with bipolar disorder, such as long-term cognitive changes as well as structural changes apparent on imaging. While we have no direct way of knowing if some of the genes we captured with our screen are involved or not in setting in motion such long-term changes, it is to be noted that some of these gene changes have also been reported in postmortem brains of bipolar disorder, schizophrenia, and dementia patients (Table 7), presumably affecting cognition. Moreover, we have candidate genes in our data set with roles in brain infrastructure, including neurotrophic, cell death, and myelin-related genes (Table 5). More chronic treatments should, nevertheless, be pursued to verify and expand the findings presented in this paper.

Different combinations of stimulants and mood stabilizers could be used in a comprehensive functional pharmacogenomic approach such as we have described. They could conceivably lead to different results, which would be interesting and welcome, since it is unlikely we are capturing with our model the full spectrum of gene expression changes and mechanisms. However, if those drug combinations indeed mimic and modulate the same core phenomenology, the Venn diagrams of the overlap between different drug treatments will be of high interest in terms of identifying the key molecular players involved in the effects, as opposed to those involved in the (very different) side effects of the individual drugs.

It is to be noted that our experimental approach for detecting gene expression changes relies on a single methodology, Affymetrix GeneChip oligonucleotide microarrays. It is possible that some of the gene expression changes detected from a single biological experiment, with a one-time assay with this technology, are biological or technical artefacts. With that in mind, we have designed our experiments to minimize the likelihood of having false positives, even at the expense of having false negatives. Working with an isogenic mouse strain affords us an ideal control baseline of saline-injected animals for our drug-injected animals. We performed six independent *de novo* biological experiments, at different times, with different batches of mice (Figure 1b). We have pooled material from the first three experiments, and carried out microarray studies. We have then pooled material from the next three experiments and carried out a second set of microarray studies. The pooling process introduces a built-in averaging of signal. We used the Venn diagram approach and only considered the genes that were reproducibly changed in the same direction in both microarray experiments. This overall design is geared to factor out both biological and technical variabilities. It is to be noted that the concordance between reproducible microarray experiments using the latest generations of oligonucleotide microarrays and other methodologies such as quantitative PCR, with their own attendant technical limitations, is estimated to be over 90%.[85] Moreover, our approach, as described above, is predicated on the existence of three internal crossvalidators for each gene that is called reproducibly changed: (1) is it changed by the other drug also, (2) is the change prevented by cotreatment with both drugs, and (3) is it changed in multiple brain regions, all of which are independent microarray experiments.

We did not see in the mouse work described in this report some of the changes that we had previously reported in rat using a similar, methamphetamine only, paradigm.[6] While some of this may be technical, that is, the mouse U74v2 A and B chips that we used did not have probe sets for some of our top findings in the previous report such as GRK3 (G-protein coupled receptor kinase 3), there are genes that are present in both the rat and mouse chips, where we see consistent changes in one species but not the other. The clock gene DBP (D-box-binding protein), for example, showed changes in rat, but a DBP-related EST did not show changes in our mouse experiments. However, we do see changes in mouse in MOP3/BMAL1, which is upstream of DBP in the same pathway. Conversely, PENK was changed in mouse and not in rat. However, a related peptide in the same pathway, prodynorphin, was marginally changed in rat (AB Niculescu III and R Kuczenski, unpublished data). While clearly technical (experimental methodology, drug doses, pharmacokinetics) and biological (interstrain, interspecies) differences remain open questions deserving future extensive comparative work, it may be that in similar paradigms across different species, it is pathways and mechanisms rather than individual players that are more conserved. That would in turn imply that a convergent functional genomics approach such as ours, where one crossmatches animal gene expression changes with human linkage data at an individual gene level, productive as it may be, could miss many things. An arguably better approach, awaiting more complete

Table 8 Candidate genes in our data sets encoding targets of existing pharmacological agents

Accession Number	Symbol - Description	Brain Region (Drug-Category) Fold Change	No. of lines of evidence	Family	Drug
U60150	VAMP2 - vesicle-associated membrane protein 2 (synaptobrevin 2)	AMY (VPA-III) 0.71/ 0.5 CP (VPA-IV) 1.74/ 6.06	4/6		botulism toxin
J04192	CHRM1 - cholinergic receptor, muscarinic 1	CP (VPA-IV) 1.52/ 3.03	3/6	Ion channel	ipratropium, olanzapine, tolterodine
AJ238309	DAT1 - (SLC6A3) - solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	VT (METH-IV) 1.41/ 1.74	3/6	transporter	amphetamine, modafinil, sibutramine, venlafaxine
U32329	EDNRB - endothelin receptor type B	CP (METH-III) 1.52/ 1.41	2/6	G-protein coupled receptor	bosentan
U14420	GABRB3 - gamma-aminobutyric acid (GABA) A receptor, beta 3	CP (VPA-IV) 1.41/ 14.9	2/6	Ion channel	lorazepam, olanzapine, sevoflurane, zaleplon, zolpidem
M62374	GABRG2 - gamma-aminobutyric acid (GABA) A receptor, gamma 2	CP (VPA-IV) 1.23/ 1.52	2/6	Ion channel	lorazepam, olanzapine, sevoflurane, zaleplon, zolpidem
M63685	5HTR2C - 5-hydroxytryptamine (serotonin) receptor 2C	CP (METH-IV) 1.23/ 10.56	2/6	G-protein coupled receptor	mirtazapine, nefazodone, olanzapine, quetiapine, risperidone, ziprasidone

Ingenuity pathway analysis (Ingenuity, Mountain View, CA, USA) was used to identify genes in our data sets that are targets of existing pharmacological agents. Meth: methamphetamine; VPA: valproate; PFC: prefrontal cortex; AMY: amygdala; CP: caudate putamen; NA: nucleus accumbens; VT: ventral tegmentum. Roman numerals in the brain region data column represent the category of the gene.

data sets as well as more sophisticated bioinformatics tools now emerging, would be to do such a cross-matching at a pathway and mechanism level.

Conclusions and future directions

The results presented in this paper have a series of direct implications. First, in terms of pharmacotherapy and drug development, some of the candidate genes in our data set encode for proteins that are modulated by existing pharmacological agents (Table 8), which may suggest future avenues for rational polypharmacy using existing agents. Moreover, our data sets of the effects of methamphetamine and valproate on gene expression in different key brain regions (Tables 1–3) may be used as a source of new targets for drug development. Individual genes involved in the response to methamphetamine could be of relevance for developing faster acting antidepressant agents, in addition to agents for the treatment of stimulant drug abuse. Individual genes involved in the response to valproate may be of relevance for developing next-generation mood-stabilizing agents, antiseizure agents, as well as in pharmacogenetic and pharmacoimaging testing of responders *vs* nonresponders.

Second is the uncovered relationship between genes involved in pain response and candidate genes for mood. The clinical literature has long abounded in examples of somatic pain complaints in depressed patients, and the use of antidepressants and anticonvulsant mood stabilizers to treat pain.[86] It seems possible that nature has recruited more primitive mechanisms related to pain perception for higher functions such as mood.[87] The utility of regulating pain thresholds in relationship to ones' moods (increased threshold in elevated mood, decreased threshold in depressed mood) is of speculative evolutionary interest, and of pragmatic clinical importance. Specifically, treating mood disorders proactively with pain-regulating agents, and pain disorders with mood-regulating agents, warrants pursuit at the level of both drug development and clinical trials.

Third, the model that emerges out of the GO analysis of our data is that of environmental stimuli leading to changes in cell communication and infrastructure changes, and those in turn leading to behavioral outputs (Figure 5b). The cybernetic-like simplicity of the model should not overshadow the important fact that it is the result of the empirical coalescence of data in a nonhypothesis-driven,

discovery-type approach. Moreover, the implications for understanding the pathophysiology and treatment of bipolar and related disorders are profound. One needs to modulate environmental input, internal cell communication and infrastructure, and behavioral output, in the treatment of these disorders. It is a place where pharmacotherapy and cognitive–behavioral therapy can and should go hand in hand.

In conclusion, we propose that our comprehensive Convergent Functional Genomics approach is a useful starting point in helping unravel the genetic code and neurobiology of bipolar and related disorders, and generates a series of leads for both future research and clinical practice.

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Competing interest statement

The authors declare that they have no competing financial interests.

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