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ARTICLE

Altered gene expression in mice treated with the mood stabilizer sodium valproate

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Abstract

Valproate is now the most widely prescribed mood-stabilizing drug and is being used increasingly in the treatment of bipolar disorder. However, the mechanism of action for valproate remains unclear. Microarray analysis was used to identify genes and cellular pathways that are affected in the mouse brain after treatment with valproate at human therapeutic concentrations. This study has identified 11 genes that are differentially expressed by $\geqslant$2-fold when compared to control untreated mice. Altered expression of four of these genes was also validated by quantitative PCR analysis. Valproate was found to significantly decrease the expression of zinc finger protein of the cerebellum 1 (ZIC1) and increase the expression of Scm-related gene containing four mbt domains (SFMBT2), structural maintenance of chromosome 4-like 1 (SCM4L1), and prostate apoptosis response-4 (PAR-4). Many of the genes identified are involved in the development and function of the brain. These results indicate that valproate regulates a large number of different functional pathways in the brain. Understanding the molecular and cellular mechanisms by which valproate achieves its therapeutic action represents a valuable step in clarifying the pathophysiology of bipolar disorder.

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Introduction

Bipolar disorder is a psychiatric illness affecting ~1–2% of the population worldwide. Despite well-established genetic diatheses and extensive research, the biochemical abnormalities underlying the predisposition to and the pathophysiology of bipolar disorder remains to be clearly established.

Valproate is now the most widely prescribed anti-epileptic drug and is being used increasingly in the treatment of bipolar disorder (Mitchell and Malhi, 2002). However, the mechanism of action for valproate remains unclear. Although the acute pharmacological effects of valproate treatment are well established, its anti-manic action is only seen after at least 1 wk of treatment. The acute effects of valproate have been investigated by using rat hippocampal slice patch-clamp electrophysiology (Martin and Pozo, 2004). In this study, valproate was shown to decrease the amplitude of excitatory post-synaptic currents (EPSC), without modifying inhibitory post-synaptic currents. Valproate also induced a significant reduction of the non-NMDA EPSC component, without modifying the NMDA EPSC component (Martin and Pozo, 2004). Furthermore, studies by Cunningham and colleagues identified that valproate could potentiate post-synaptic responses by interaction with the benzodiazepine regulatory site of the $GABA_A$ receptor (Cunningham et al., 2003).

Extensive research over the last 10 yr has identified a number of proteins and pathways that are affected by valproate treatment. It is well established in the literature that valproate directly inhibits the glycogen synthase kinases (GSK)-3α/β (Klein and Melton 1996). This direct inhibition occurs by a mechanism in which

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valproate increases the inhibition-associated phosphorylation of GSK-3β on Ser9 (Sarno et al., 2002). This mechanism has also been demonstrated for Akt (protein kinase B), in which valproate also increases the activation-associated phosphorylation of Akt on Ser473 (Sarno et al., 2002). It has also been identified that GSK3-β is inhibited by lithium (Jope, 2003) and clozapine (Kang et al., 2004). In several studies, valproate has been shown to increase the expression of chaperones that assist in the folding of proteins in the endoplasmic reticulum including GRP78, GRP94, calreticulin, and the cytosolic chaperone HSP70 (Bown et al., 2000; Corson et al., 2004; Wang et al., 1999). These chaperone proteins can bind Ca^{2+} in the endoplasmic reticulum and protect cells from the deleterious effects of damaged proteins.

One of the most substantial findings is that valproate increases Bcl-2 expression (Yuan et al., 2001). Bcl-2 is a protein that regulates the ERK pathway (Hao et al., 2004). Several studies have shown that valproate also activates the ERK pathway and induces ERK pathway-mediated neurotrophic action (Hao et al., 2004; Yuan et al., 2001). Another common finding is that valproate affects the phosphoinositol cycle. Rats treated with valproate exhibited a significant decrease in myoinositol concentration (Harwood and Agam, 2003; O'Donnell et al., 2003). This is possible because the brain is highly sensitive to inositol depletion as the blood–brain barrier limits the availability of plasma inositol, making it dependent on inositol recycling and synthesis (Williams et al., 2002).

Among other studies investigating valproate action, it has been demonstrated that valproate increases the expression of 5-lipoxygenase and the acetylation of histone H3, which is involved in chromatin remodelling (Yildirim et al., 2003). Moreover, valproate has been shown to increase AP-1 expression (Chen et al., 1999a), and increase thyrotrophin-releasing hormone (TRH), TRH receptor and TRH-like peptide levels within most brain regions (Pekary et al., 2004).

It is clear from the large number of studies that valproate affects and alters the expression of a large group of genes spanning a number of different cellular pathways. Many of the actions of valproate are shared by other mood-stabilizing agents, including lithium and carbamazapine, as well as common antipsychotics such as olanzapine and clozapine (Bai et al., 2004; Williams et al., 2002).

The present study describes the analysis of altered gene expression in the whole mouse brain after treatment with sodium valproate at human therapeutic concentrations. Identifying the molecular and cellular mechanisms by which sodium valproate achieves its therapeutic action represents a valuable step in clarifying the pathophysiology of bipolar disorder.

Methods

Animals

Male C57BL/6 mice (ARC, Perth, Australia) were used for all experiments. These animals were maintained with standard chow and water ad libitum during the standard 1 wk initial quarantine and treatment periods in a controlled temperature and humidity environment with a 12 h day/night cycle. The animal studies were approved by the Garvan Institute of Medical Research/St Vincent's Hospital Animal Experimentation Ethics Committee (NSW, Australia). All animals were weighed before each injection and were 8–10 wk old at the commencement of each study.

Animal treatment and serum level evaluation

Littermate mice received intraperitoneal injections once daily of 10 ml/kg of sterile 0.9% saline solution for 7 d. Sodium valproate (2-propylpentanoic acid, Sigma-Aldrich, Sydney, Australia) was administered via intraperitoneal injection at a rate of 350 mg/kg . d for 7 d. Valproate was prepared in 0.9% saline solution. Four hours after the last injection on day 7 of treatment, mice were euthanized under 4% halothane anaesthesia. The whole brain was immediately removed, snap frozen in liquid nitrogen and stored at $-80\ ^{\circ}C$ until required. In addition, a cardiac puncture procedure was used to collect 1 ml of blood for drug serum quantitation. Blood samples from each mouse were assayed for valproate serum concentration by the SydPath Diagnostic Testing Facility (St Vincent's Hospital, Sydney, Australia).

Preparation of total RNA

Total RNA was prepared by extracting whole mouse brain tissue in TRI Reagent (Sigma-Aldrich). Thawed whole brains were homogenized in 3 ml TRI Reagent solution for 10 min using a polypropylene hand homogenizer (Sigma-Aldrich). After homogenization, 600 μl chloroform (Sigma-Aldrich) was added and mixed by vortexing for 30 s. After 10 min incubation, the upper inorganic phase was separated by centrifugation at 12 000 *g* (10 500 rpm) for 10 min at 4 $^{\circ}C$. Total RNA was precipitated by adding 1.5 ml isopropanol. Total RNA pellets were washed with 2 ml of 75% ethanol, air dried in a fume hood and resuspended in 100 μl RNase-free water. The concentration of total RNA samples were determined by

Table 1. Differential gene expression for valproate-treated mice vs. control mice by microarray analysis

Affymetrix code	Gene name	Gene symbol	Accession number	Δ low	Δ high
Decreased expression in mice treated with valproate					
102773_at	Carbonic anhydrase 8	CAR8	X61397	6.1	4.0
M12481_5_at	Actin, beta, cytoplasmic	ACTB	M12481	3.0	–
92802_s_at	Myelin proteolipid protein	PLP	M16472	2.7	–
160614_at	Phosphatase and tensin homologue	PTEN	U92437	2.6	–
104169_at	Zinc finger protein of the cerebellum 1	ZIC1	BC063247	2.5	–
103787_at	Potassium voltage-gated channel, subfamily A member 1	KCNA1	Y00305	2.5	–
101451_at	Paternally expressed 3	PEG3	AF038939	2.4	–
92673_at	SH3-domain GRB2-like 2	SH3GL2	U58886	2.1	–
Increased expression in mice treated with valproate					
100706_f_at	SCM-like with four mbt domains 2	SFMBT2	AA407367	2.5	2.2
101906_at	Structural maintenance of chromosomes 4-like 1	SMC4L1	AA032310	2.2	2.0
93439_at	Prostate apoptosis response-4	PAR-4	AA260005	2.8	–

measuring the absorbance at 260 nm using a GeneQuant UV spectrophotometer (Pharmacia Biotech, Sydney, NSW).

Target preparation and microarray hybridization

Individual total RNA samples were selected from 45 mice that were grouped as follows. Fifteen control mice were selected (treated with saline solution only). Fifteen mice with a valproate serum level between 32–35 mg/l (designated as low-valproate). Fifteen mice with a valproate serum level between 42–91 mg/l (designated as high-valproate). Within each group, five individual mouse total RNA samples were randomly selected and pooled together to form three separate pooled samples. Microarray biotinylated copy RNA (cRNA) probes were prepared using the BioArray High Yield RNA Transcription labelling kit (Affymetrix, Santa Clara, CA, USA) following the manufacturer's instructions. Microarray cRNA probes were prepared for each separate pooled sample in all three groups (control, low- and high-valproate). These nine cRNA probes were first hybridized to Affymetrix Genechip Test 2 microarrays. The quality of each sample was assessed by examining the 3′/5′ ratios for mouse housekeeping genes. Following quality-control assessment, cRNA probes were hybridized to Affymetrix GeneChip® Murine Genome U74Av2 microarrays following the protocol outlined in the Affymetrix GeneChip® Expression Analysis Technical manual. Microarrays were washed using a semi-automated GeneChip® Fluidics Station 400 (Affymetrix) and scanned using a Agilent GeneArray Scanner (Agilent Technologies, Palo Alto, CA, USA).

Data analysis

Using Affymetrix Microarray Analysis Suite (MAS) software version 5.0, the fluorescent signal intensity of all 12 450 transcripts contained on the Affymetrix GeneChip® Murine Genome U74Av2 microarray chips were determined. The quality of hybridization was assessed by examination of the microarray hybridization image, and 3′/5′ ratios of the mouse housekeeping genes GAPDH (M32599) and β-actin (M12481). The raw intensity values were normalized to the mean intensity of all probe sets. Genes were initially filtered for a 'present' call as assigned by the Affymetrix software for each of the hybridized microarray chips. Genes were selected for further analysis only if they were called as 'present' on all nine microarrays (3 control, 3 low-valporate, 3 high-valproate). Differentially expressed genes were determined by calculating the mean signal intensity for each gene in each group (control, low- and high-valproate). The fold change signal ratio was then calculated for control vs. treatment groups (low- and high-valproate) and used to rank genes based on the magnitude of fold difference. Genes were further selected that had a $\geqslant$2-fold difference between control and treatment groups (Table 1).

Reverse transcription

Prior to reverse transcription, total RNA was incubated for 15 min at room temperature with 2 U of DNase I (Invitrogen, Sydney, Australia) in 1 × DNase I reaction buffer (Invitrogen) to remove any genomic DNA contamination. DNase I treatment was

Table 2. Quantitative PCR oligonucleotide primers used to amplifying specific genes using mouse brain cDNA

Gene	Forward primer	Reverse primer	Size (bp)	An. (°C)
GAPDH	5′-AACTTTGGCATTGTGGAAGGG-3′	5′-TCATCATACTTGGCAGGTTTCTCC-3′	272	58
CAR8	5′-GCTTAGTGTTTCCTGATGCTA-3′	5′-CTCCTGACAAGACTGATTTTG-3′	192	55
ACTB	5′- TGGGAATGGGTCAGAAGGACTC-3′	5′- GGTCATCTTTTCACGGTTGGC-3′	227	58
PLP	5′-GTGTTGTGCTAGATGTCTGGT-3′	5′-GAAAGAGGCAGTTCCATAGAT-3′	220	55
PTEN	5′-GGATGGATTCGACTTAGACTT-3′	5′-CAAAAGGATACTGTGCAACTC-3′	218	55
ZIC1	5′-GTTCAGAGAACCTCAAGATCC-3′	5′-TGTAGGACTTATCGCACATCT-3′	171	55
KCNA1	5′-AGTATCCCCGATGCTTTC-3′	5′-GGTCACTGTCAGAGGCTAAG-3′	250	58
PEG3	5′-TTCCAGACCACAGAGGTTG-3′	5′-AGAGGTAGGGGACAGAACAC-3′	309	58
SH3GL2	5′-TAAGACAAGCTTCATCTCAGC-3′	5′-CAGGTTCCAAGTCATACAGAG-3′	180	55
SFMBT2	5′-TTGCTAAAGACTGACTCAAGG-3′	5′-ACCTTCTAGGAATGGTTTCTG-3′	195	55
SMC4L1	5′-CTGAGCCTTAGTCATCAAAAG-3′	5′-GCTTCTTAAAACTAGGGTGGT-3′	225	55
PAR-4	5′-AATAGATATCCCCGAACAGAC-3′	5′-TCATCTTCCATGTCATCTAGG-3′	245	55

terminated by adding 2 μl of 25 mM EDTA (pH 8.0) and incubated at 65 °C for 10 min. Reverse transcription was performed using 2 μg total RNA. Total RNA was denatured for 10 min at 65 °C in the presence of oligo $dT_{12–18}$ (500 ng, Invitrogen). After denaturing, cDNA was synthesized in a 50 μl reaction volume containing 1 × First Strand buffer (Invitrogen), dNTP (400 μM each, Promega, Sydney, Australia), DTT (10 mM), RNaseOUT™ ribonuclease inhibitor (80 U, Invitrogen), Superscript™ III M-MLV reverse transcriptase (400 U, Invitrogen) and incubated for 60 min at 50 °C. The reaction was terminated by incubation at 70 °C for 15 min and stored at −20 °C until required.

Quantitative PCR

The differential gene expression detected using microarray analysis was validated using quantitative PCR. For quantitative PCR amplification, 1 μl of 2/5 diluted cDNA was added to a 10 μl PCR reaction containing 1 × Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen) and forward and reverse primers (200 nM each). PCR primers were designed using MacVector version 6.5.3 software (Accelrys, Cambridge, UK) and synthesized by Sigma Genosys (Sydney, Australia) (Table 2). Amplification conditions included the following; uracil–DNA glycosylase treatment at 50 °C for 2 min, denaturation at 95 °C for 2 min, and 45 cycles of amplification (denaturation at 95 °C for 5 s, annealing for 15 s, and extension for 15 s at 72 °C). SYBR Green I fluorescence intensity was measured at the end of the annealing step. Following amplification, samples were dissociated by incremental heating between 72 °C and 99 °C, at a rate of 0.2 °C/s. During this dissociation, SYBR Green I fluorescence was constantly measured. Amplification was performed in 100 μl tubes (Corbett Research, Sydney, Australia) using a Rotor-Gene 3000 PCR machine (Corbett Research).

Quantitative PCR critical threshold (C_t) values for each sample were determined using Rotor-Gene version 5.0.37 software (Corbett Research). The number of mRNA copies for each gene in each sample was calculated by including in each PCR experiment, seven serial diluted DNA standards of known concentration. PCR amplification was performed simultaneously on eight individual mouse cDNA samples prepared from control mice and eight individual mouse cDNA samples prepared from mice treated with valproate.

The relative expression for each gene of interest (GOI) was expressed as a ratio of the number of GOI mRNA copies, to the number of copies of GAPDH mRNA for each sample. The GOI:GAPDH mRNA ratio mean and standard deviation for control and valproate cDNAs were calculated using StatView software version 5.47 (Abacus Concepts, Berkeley, CA, USA). An unpaired Student's t test was used for analysis of PCR quantification results. A two-tailed p value of <0.05 was considered statistically significant.

Results

Valproate treatment

A total of 49 mice were treated with 350 mg/kg . d of valproate for 7 d. Their blood valproate levels ranged from 30–91 mg/l with a mean of 40.4 ± 10.8 mg/l (mean ± S.D.). Thirty mice with the highest valproate blood concentration were selected for microarray analysis. These mice had a blood valproate concentration ranging from 32–91 mg/l. These 30 mice were

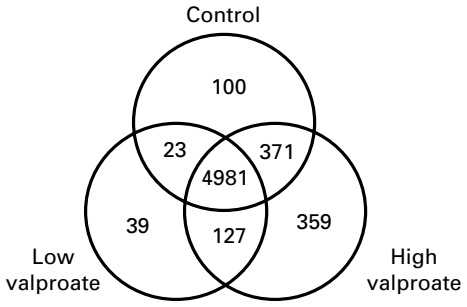

Figure 1. Venn diagram illustrating the overlap in whole brain mRNA expression observed in control mice, and mice treated for 7 d with valproate. Treated mice were grouped by low (32–35 mg/l) and high (42–91 mg/l) sodium valproate serum concentrations.

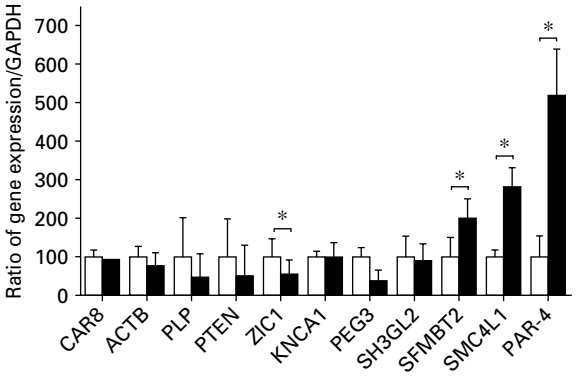

Figure 2. Steady-state mRNA levels of genes altered after treatment of mice with valproate. The relative expression level for each gene has first been normalized to GAPDH expression, and then the level of expression in control samples has been normalized to 100 (* $p < 0.05$). □, Control; ■, valproate.

then grouped (15 in each group) into low-valproate (32–35 mg/l) and high-valproate (42–91 mg/l) groups.

GeneChip analysis and altered gene expression

Pooled RNA samples from the three treatment groups (control, low- and high-valproate) were prepared and hybridized to Affymetrix U74Av2 microarrays. Of the 12 450 transcripts contained on the Affymetrix U74Av2 microarrays, a total of 4981 transcripts were expressed in common (called as present) across all nine microarray hybridizations (Figure 1). In general, $>90\%$ of all genes expressed showed common expression across the three treatment groups (control, low- and high-valproate). Data analysis was performed on these 4981 transcripts to identify genes with $\geqslant$2-fold altered expression between control and treatment groups. A total of 11 genes were identified as differentially expressed using $\geqslant$2-fold as the selection criteria (Table 1). Of these, eight genes were down-regulated and three genes were up-regulated by valproate treatment. The majority of genes altered by $\geqslant$2-fold difference (7/8) occurred in animals with a low valproate blood serum level (32–35 mg/l). Only one gene, carbonic anhydrase 8 (CAR8) was also significantly down-regulated by $>$2-fold difference in animals with a high valproate serum level (42–91 mg/l). Two of the three genes that were up-regulated by valproate treatment, namely SCM-like with four mbt domains 2 (SFMBT2) and structural maintenance of chromosomes 4-like 1 (SMC4L1) were also significantly up-regulated in the brains of mice with a higher valproate blood serum level.

Verification of gene expression by quantitative PCR

The validity of the 11 differentially expressed genes was evaluated by quantitative PCR analysis. A total of eight control brain RNA samples (saline treatment) and eight treated brain RNA samples (valproate) were selected and used as template to perform quantitative PCR analysis. The change in expression (up- or down-regulation) was confirmed by quantitative PCR analysis for all 11 genes examined (Figure 2). However, only four genes, Zinc finger protein of the cerebellum 1 (ZIC1), SFMBT2, SMC4L1 and prostate apoptosis response-4 (PAR-4) showed statistically significant altered gene expression ($p < 0.05$).

Discussion

This study has used an animal model of valproate treatment to investigate altered gene expression in the mouse brain. The utilization of an in-vivo model of drug action has some advantages over in-vitro cell culture based drug treatment models. In-vitro studies have used cell lines including human SY5Y neuroblastoma (Chen et al., 1999a), rat C6 glioma cells (Chen et al., 1999a), human heptocarcinoma HepG2 (Kim et al., 2004), PC12 (Cordeiro et al., 2004), yeast cells (*Saccharomyces cerevisiae*) (Vaden et al., 2001), and the amoebae *Dictyostelium discoideum* (Williams et al., 2002). By using an in-vivo system, the effect of valproate treatment on gene expression profiles can be observed in a functional brain. In addition, animal models also allow behavioural studies to be performed. Cell culture-based studies are unable to replicate the feature that as many as 20 different cell types are present in the brain, as well as the dynamic interactions between neurons themselves, and with other cells. The treatment of rodents with human

therapeutic drugs such as valproate has been performed by other groups. The majority of these studies have used rats (Chen et al., 1999b; Hao et al., 2004; Sands et al., 2000; Shaltiel et al., 2004; Stout et al., 2001; Wang et al., 1999), while only a few have used mice (Faiella et al., 2000; Yildirim et al., 2003). Each study has used a different experimental design, with one study adding valproate to the chow feed (Hao et al., 2004) and others using intraperitoneal injections once or twice a day (Chen et al., 1999b; Wang et al., 1999). Also, the duration of treatment ranged from 1 d for acute studies (Chen et al., 2000) and up to 6 wk for chronic studies (Hao et al., 2004). Only four studies measured the blood serum level of valproate at the time of sacrifice (Chen et al., 1999b; Hao et al., 2004; Sands et al., 2000; Stout et al., 2001). Mice are particularly advantageous for functional studies because of the large number of knockout and transgenic mouse lines already developed.

Microarray technology represents an increasingly powerful and popular tool for identifying genes associated with human mental disorders (Bunney et al., 2003). Microarray technology has been used to investigate how common human psychotropic drugs work, in an attempt to identify molecular targets for new types of drugs (Kontkanen et al., 2002). More recently, microarray technology has been used to identify the individual genes altered by valproate treatment (Jurata et al., 2004). In this study, Jurata et al. examined gene expression in human post-mortem samples from normal controls and patients with bipolar disorder, and in human neuroblastoma flat (NBFL) cells treated with valproate. Among the list of genes identified, dopa decarboxylase, dopamine β-hydroxylase, and dihydropyrimidinase-related protein 3 were found to be decreased in NBFL cells after valproate treatment, and the gene spinocerebellar ataxia 7 was increased in bipolar disorder patients (Jurata et al., 2004).

After data analysis of the microarray hybridizations in our own study, it was noted that the majority of genes (82%) altered by $\geqslant$2-fold difference were found in mice with a lower blood valproate level. Out of the list of 11 genes (Table 1), only three genes CAR8, SFMBT2, and SMC4L1 were also differentially expressed in mice with a higher blood valproate level. This is an interesting finding, and may suggest that valproate has differing mechanisms of action, depending on the serum concentration achieved. The low (32–35 mg/l) and high (42–91 mg/l) valproate serum concentrations obtained in this study were selected to approximate subtherapeutic/marginally therapeutic and clearly therapeutic levels. There is minimal data on therapeutic concentration ranges of valproate in the acute treatment of mania (Mitchell, 2001). In the only data from a randomized controlled trial, Bowden and colleagues found that concentrations of at least 45 mg/l were associated with a greater likelihood of response, while levels of 125 mg/l or more were likely to cause toxicity (Bowden et al., 1996). However, as there are no other such studies of therapeutic valproate concentrations, and specifically no studies in which randomization to particular concentration ranges has been undertaken, some uncertainty over the specific effective range of valproate continues. Further experiments that include longer and shorter treatment periods at differing doses are required to fully examine this finding.

Using quantitative PCR analysis, four of the 11 genes identified by microarray analysis were confirmed as being significantly differentially expressed. However, the change in expression for the other seven genes identified by microarray analysis was not independently confirmed by quantitative PCR. Each microarray hybridization consisted of a pool of five individual whole mouse brain RNA samples, with each sample contributing equally to the total sample. This pooling technique equilibrated any small fluctuations in gene expression between individual mice that were pooled onto the same microarray (Peng et al., 2003). For each treatment group (control, low- or high-valproate) a total of three independent microarray hybridizations were performed. The three microarray hybridizations represent the cumulative gene expression data from 15 individual mice. For quantitative PCR analysis, amplification was performed on individual mouse brain samples. There was more variability in gene expression observed using quantitative PCR analysis than microarray analysis on pooled samples (data not shown). This explains why only four of the 11 genes identified by microarray analysis were confirmed as statistically significantly altered in gene expression between control and valproate treatment.

This study has identified and confirmed four genes whose expression levels are significantly altered in the brains of mice treated with sodium valproate, namely ZIC1, SFMBT2, SCM4L1 and PAR-4. ZIC1 is a member of a family of proteins (ZIC1-5) that share five highly conserved tandem repeats known as C2H2 zinc finger (ZF) motif (Aruga, 2004). Studies in mice have shown that ZIC1 transcript is the most abundant throughout development, particular in postnatal cerebellum (Nagai et al., 1997). In knockout studies, ZIC1$^{-/-}$ mice generally died within 1 month of birth, but some lived as long as 8 wk (Aruga et al., 1998). ZIC$^{-/-}$ mice experienced behavioural abnormalities, including a

disturbed gait that caused the mice to drag their hindlimbs behind them and an inability to walk in a straight line. Cerebellar abnormalities were also found in ZIC1$^{-/-}$ mice. Macroscopically, the most remarkable change found in the central nervous system (CNS) was hypoplasia of the cerebellum. Considering that ZIC1 is important in the development and function of the brain, the dysregulation of ZIC1 may be an important finding and may be a potential mechanism of valproate action. Whether valproate acts directly on ZIC1, or via an upstream mediator remains to be elucidated. It is also interesting to note that the ZIC1 gene, which in humans is found on chromosome 3q24, lies next to the bipolar disorder susceptibility locus identified at 3q25-q26 (Badenhop et al., 2002).

Scm-related gene containing four mbt domains (SFMBT2) contains two kinds of characteristic homology domains, the SPM domain and mbt repeats (Usui et al., 2000). The SPM domain was first described in the *Drosophila* Scm gene product, and named from the three *Drosophila* proteins, Scm, ph, and l(3)mbt (Bornemann et al., 1996). The mbt repeats were originally reported in the *Drosophila* l(3)mbt gene, whose recessive mutations are associated with malignant transformation of the neuroblast and ganglion-mother cells in the larval brain (Wismar et al., 1995).

To date, only four genes [l(3)mbt, Scm, SCML2, and Sfmbt] have been reported to contain the mbt repeats. The *Drosophila* Scm and l(3)mbt work as a member of the transcriptional repressor PcG genes and a tumour suppressor gene respectively. Functional importance has also been suggested for the human mbt, which may be involved in the proper progression of cell division (Koga et al., 1999). However, most mammalian genes in this family await further intensive functional studies. Considering SFMBT2 is a member of a recently identified gene family, it is difficult to hypothesize the functional role SFMBT2 has in relation to valproate action. There is some evidence that proteins which contain the same mbt functional domains as SFMBT2 are involved in the development of the brain (Wismar et al., 1995), which would suggest as with ZIC1, that valproate may act upon genes involved in brain function and development.

The structural maintenance of chromosome (SMC) genes were originally identified in *Saccharomyces cerevisiae* as genes required for proper condensation and segregation of mitotic chromosomes (Strunnikov et al., 1993). *Xenopus* chromosome-associated protein-C (XCAP-C) and XCAP-E, the SMC homologues in *Xenopus* isolated from mitotic oocyte extracts, were shown to be required for the early stages of mitotic chromosome condensation in vitro and to be physically associated with condensed chromosomes as part of a multiprotein complex called 'condensins' (Hirano et al., 1997). These studies revealed that the SMC family proteins are integral components of the machinery that modulates chromosome structure for mitosis. Although studies have highlighted the functional significance of the SMC protein family, it remains unclear how their functional specificities are determined, or the molecular mechanisms with which SMC functions in chromosome condensation (Schmiesing et al., 1998). As the majority of neuronal cells in the brain do not divide, the specific functional consequence of SMC4L1 up-regulation by valproate is unclear.

PAR-4 was first identified by differential screening for early response genes up-regulated during induction of apoptosis in prostate cells (Sells et al., 1994). PAR-4 is known to interact with the product of the Wilms' tumour gene (WT1), the atypical protein kinase C-ξ, and the death-activated protein-like kinase (Dlk) (Diaz-Meco et al., 1996; Johnstone et al., 1996; Page et al., 1999). WT1 is involved in the transcriptional regulation of the Bcl-2 gene and has been shown previously to bind to two different sites on the Bcl-2 promoter to both repress and activate the transcription of this gene (Mayo et al., 1999). Atypical protein kinase C-ξ is involved in a number of important cellular functions, including cell proliferation (Diaz-Meco et al., 1996) and Dlk participates in regulation and coordination of mitosis and cytokinesis (Page et al., 1999). Studies have shown that PAR-4 is a WT1-interacting protein that modulates the transcriptional activities of WT1 via physical interactions (Johnstone et al., 1996). It is known that valproate, lithium, clozapine, and olanzapine increase Bcl-2 expression in neuronals cells (Bai et al., 2004; Chen et al., 1999b). It may be possible that the increase in Bcl-2 protein observed after valproate treatment is a downstream effect of up-regulation of the PAR-4 gene. The up-regulation of PAR-4 would transcriptionally down-regulate WT1, thus inhibiting WT1's ability to transcriptionally repress Bcl-2 expression (Johnstone et al., 1996). It remains unclear how, or why valproate regulates PAR-4 expression. Also, apart from Bcl-2 anti-apoptotic functions, the consequence of increased Bcl-2 expression remains to be investigated. In addition, it is worth noting that in humans, PAR-4 is found on chromosome 12q21.2, which is situated next to a bipolar disorder susceptibility locus identified at 12q23–q24 (Morissette et al., 1999).

In conclusion, this study has identified two genes, ZIC1 and SFMBT2 whose expression is altered by valproate and are involved in the development of the

brain. As yet, it is unclear what the significance of altered SMC4L1 expression on brain function is. It is coincidental that SMC4L1 and one of the genes that PAR-4 interacts with, Dlk are both involved in mitosis. The well-established finding that a number of psychotropic medications such as valproate, lithium, clozapine, and olanzapine increase Bcl-2 expression may be a result of the up-regulation of the upstream pathway protein, PAR-4.

Further investigation is required to confirm the altered gene expression identified in this study. This would include quantitative PCR analysis on post-mortem brain tissue from drug-naive bipolar disorder patients, valproate-treated bipolar disorder patients and controls. Such analysis would enable comparison of the expression between an animal model of valproate treatment and tissue from affected bipolar disorder patients. In addition, it would be interesting to investigate, using in-situ techniques, the anatomical expression of these genes in this mouse model and human bipolar disorder brain-tissue specimens.

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Statement of Interest

None.

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