

Effect of Divalproex on Brain Morphometry, Chemistry, and Function in Youth at High-Risk for Bipolar Disorder: A Pilot Study

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Abstract

Objective: Divalproex has been found efficacious in treating adolescents with and at high risk for bipolar disorder (BD), but little is known about the effects of mood stabilizers on the brain itself. We sought to examine the effects of divalproex on the structure, chemistry, and function of specific brain regions in children at high-risk for BD.

Methods: A total of 24 children with mood dysregulation but not full BD, all offspring of a parent with BD, were treated with divalproex monotherapy for 12 weeks. A subset of 11 subjects and 6 healthy controls were scanned with magnetic resonance imaging (MRI, magnetic resonance spectroscopy [MRS], and functional MRI [fMRI]) at baseline and after 12 weeks.

Results: There were no significant changes in amygdalar or cortical volume found over 12 weeks. Furthermore, no changes in neurometabolite ratios were found. However, we found the degree of decrease in prefrontal brain activation to correlate with degree of decrease in depressive symptom severity.

Conclusions: Bipolar offspring at high risk for BD did not show gross morphometric, neurometabolite, or functional changes after 12 weeks of treatment with divalproex. Potential reasons include small sample size, short exposure to medications, or lack of significant neurobiological impact of divalproex in this particular population.

Introduction

DIVALPROEX HAS BEEN SHOWN to be effective for the treatment of mania in adults with bipolar disorder (BD) (Bowden et al. 1994), and in open studies of children with BD (Kowatch et al. 2000; Wagner et al. 2002). Furthermore, divalproex may (Chang et al. 2003b) or may not (Findling et al. 2007) be effective in treating children with subsyndromal symptoms of BD who are at high risk for development of full BD. However, little is known about the effects of divalproex on the brain itself. Advances in neuroimaging technology, including modalities such as magnetic resonance imaging (MRI), functional MRI (fMRI), magnetic resonance spectroscopy (MRS), and diffusion tensor imaging (DTI), have allowed for *in vivo* study of such effects of psychotropic medications. Furthermore, it may be surmised that these medications act upon brain structures and circuits thought to be involved in the pathophysiology of BD. Prefrontal amygdalar circuits that regulate mood have been proposed to be

primary areas of involvement in BD (Chang et al. 2004; Strakowski et al. 2005), and abnormalities in these areas may be detected in children before the onset of fully developed BD (Chang et al. 2006). Therefore, we sought to examine the effects of divalproex on the structure, chemistry, and function of these brain regions in children at high risk for BD.

Offspring of parents with BD are at increased risk for the development of BD (Lapalme et al. 1997; Chang et al. 2003a). Such high-risk offspring with and without psychiatric symptoms have been found to have increased hippocampal volume (Ladouceur et al. 2008) and decreased cerebellar vermis *N*-acetylaspartate (NAA) (Cecil et al. 2003), although some neuroimaging studies in bipolar offspring have been relatively negative (Gallelli et al. 2005; Ladouceur et al. 2008; Singh et al. 2008). These areas are involved in mood regulation and prefrontal limbic circuitry that has been proposed as abnormal in BD. Thus, it might be surmised that with amelioration of mood symptoms changes in the neurobiological characteristics of these areas might be detected.

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Previously, we found that children with familial BD and a history of lithium or valproate exposure tended to have larger amygdalar volume than those without such exposure (Chang et al. 2005). Regarding neurochemistry, however, two MRS studies of adults with BD failed to find significant effects of valproate on brain NAA, myo-inositol (mI), or glutamate-glutamine γ -butyric acid (Glx) (Silverstone et al. 2003; Friedman et al. 2004). Regarding brain function, overall brain activation in healthy volunteer adults was increased after 14 days of valproate administration (Bell et al. 2005). At the cellular level, divalproex may have direct neurotrophic effects, including increasing prefrontal bcl-2, inhibiting glycogen synthase kinase 3B (GSK-3B), and activating the extracellular signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase pathway, all putative neuroprotective effects (Manji et al. 2000). Thus, we sought to study the neurobiological effects of divalproex in a high-risk offspring population. We hypothesized that offspring with mood and/or behavioral symptoms, but not full BD, would demonstrate increases in amygdalar volume, increases in prefrontal NAA/Creatine-phospho-creatine (Cr) ratios, and changes in prefrontal-amygdalar activation after 12 weeks of divalproex monotherapy. Because this was a pilot study with small sample sizes, we sought to generate data that would lead to hypotheses for future large-scale studies.

Methods

This protocol was approved by the Stanford University Panel of Medical Research in Human Subjects. Twenty four children with a parent with BD, who themselves had early symptoms of mood dysregulation but not full BD, were enrolled in a 12-week open label trial of divalproex monotherapy (Chang et al. 2003b). Inclusion criteria for subsyndromal subjects were age 9–18 years, a biological parent with BD I or II, and a diagnosis of “subsyndromal” BD, as defined below. Exclusion criteria were presence of a pervasive development disorder (such as autism or Asperger disorder), a neurological condition (such as a seizure disorder), a substance use disorder, intelligence quotient (IQ) less than 80, or presence of metallic implants or orthodontic braces, which would make the MRI scan not feasible.

Six healthy controls (group matched for age, IQ, and handedness with subjects from the fMRI subset) were also included in the present study. For inclusion in the control group, healthy volunteers did not have a current or lifetime *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) (American Psychiatric Association 1994) psychiatric diagnosis, had both parents without any psychiatric diagnosis by Structured Clinical Interview for DSM-IV Axis I disorders (SCID), and did not have a first- or second-degree relative with BD as determined by the Family History Research Diagnostic Criteria (Andreasen et al. 1977).³⁸

An oral and written consent from the parents as well as an oral and written assent from the adolescents were obtained, and both the parents and the offspring were interviewed. For the subsyndromal group, at least one parent had BD I or II diagnosed by the SCID (First et al. 1995), administered by a trained master's degree-level clinician and/or board-certified child and adolescent psychiatrist. For inclusion in the subsyndromal group, in addition to parental diagnosis of BD, all children either met criteria for attention-deficit/hyperactivity

disorder (ADHD), major depression, dysthymia, or cyclothymia. Additionally, subjects had to have at least moderate current mood symptoms, as indicated by a score of >10 on the Young Mania Rating Scale (YMRS) or a score of >30 on the Children's Depressive Rating Scale-Revised (CDRS-R). All subjects (patients and healthy volunteers) were evaluated by the affective disorders module of the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) (Geller et al. 1996; Geller et al. 2001), and the Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime (K-SADS-PL) (Kaufman et al. 1997). Diagnostic decisions were ultimately made by a board-certified child psychiatrist based on personal interview, discussion with the research assistant, and written notes of parental and subject responses to individual WASH-U-KSADS questions. Current and lifetime diagnoses were established according to DSM-IV criteria.

Response to treatment was defined by a week-8 score of 1 (very much improved) or 2 (much improved) on the Clinical Global Impressions Scale-Change subscale (CGI-C). A subset of 11 consecutive subjects (the last 11 enrolled in the clinical trial after funding was obtained to include MRI in the protocol) were scanned with MRI, both at baseline (pretreatment, no medications) and after 12 weeks. Furthermore, 6 healthy control subjects, matched for age, gender, and IQ, were also scanned at baseline and at 12 weeks to serve as a comparator group for fMRI.

Eleven subsyndromal subjects were scanned using morphometric MRI, ¹H-MRS, and fMRI on a 3-Tesla GE Signa scanner (Milwaukee, WI). Patients with BD had psychostimulants discontinued for at least 24 hours before the scan, primarily due to a concurrent functional MRI study of attention. They were allowed to continue any other current medications, such as mood stabilizers or antidepressants, due to the risk of mood destabilization. Medication history was obtained from direct interview with subjects and parent and review of medical records when available (Table 1).

MRI acquisition

Coronal 3D volumetric spoiled gradient echo (SPGR) series were obtained with the following parameters: time of repetition (TR)=35, time to echo (TE)=6, flip angle=45, slice thickness=1.5 or 1.6 mm, and matrix=256×192 for 124 slices. The volumetric analysis was performed using BrainImage software v. 5.3.7 (Stanford Psychiatry Neuroimaging Laboratory; <http://cibsr.stanford.edu>) for semiautomated image processing and quantification.

The processing of the scans involved removal of the non-brain tissue, correction of nonuniformity, and positional normalization to anterior and posterior commissures in a stereotactic space (Talairach and Tournoux 1988). Each brain was divided into lobes with a semiautomated stereotactic-based parcellation method (Kates et al. 1999), based on the raters' identification of the anterior commissure, the posterior commissure, and a midsagittal point above the axis created by the first two points. Raters who conducted morphometric analyses were blind to the diagnosis of each subject. Voxels comprising brain tissue were then segmented into gray matter, white matter, and cerebrospinal fluid (CSF) using a semiautomated fuzzy tissue segmentation algorithm (Reiss et al. 1998). The total brain volume (TBV) was calculated by

calculating the sum of all brain regions. Total cerebral volume was calculated by adding cerebral total tissue with cortical and ventricular CSF. Total brain tissue was calculated by adding cerebral total tissue, cerebellar tissue, and brainstem tissue.

Amygdalae were outlined manually by reliable raters (interrater reliability >0.9 with intraclass correlation coefficient) on positionally normalized brain image stacks in the coronal orientation. Amygdalae were traced starting on the slice demonstrating the thickest extent of the anterior commissure and following the structure toward the posterior end of the brain. The most superior white matter tract extending from the temporal lobe marked the inferior border, CSF marked the medial border, endorhinal sulcus marked the superior border, and a thick, central white matter tract of the temporal lobe was used as the lateral border of amygdala (Fig. 1).

Brain structure volume data were first examined for normality to conform to the assumptions of the parametric statistics employed. One-way analyses of covariance (ANCOVAs) were used for comparisons of brain structure volumes, with age and TBV as covariates. A p value of 0.05 (two-tailed) was chosen as the significance threshold.

MRS acquisition

For ^1H -MRS, a $2 \times 2 \times 2$ -cm voxel was prescribed in the right and then left dorsolateral prefrontal cortex (DLPFC), from the first axial slice above the lateral ventricles. Because slices were 5 mm thick, the voxel was placed anywhere from 0 mm to 5 mm above the lateral ventricles, immediately anterior to a line drawn between the anterior aspects of the lateral ventricles, and as far lateral as possible while remaining in the ce-

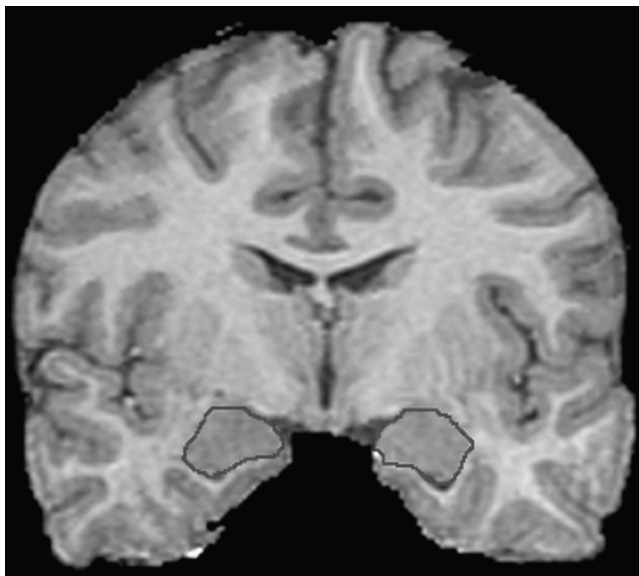


FIG. 1. Outline of the left and right amygdalae on the positionally normalized brain stack in coronal orientation. The most superior white matter tract extending from the temporal lobe marked the inferior border, cerebrospinal fluid (CSF) marked the medial border, endorhinal sulcus marked the superior border, and a thick, central white matter tract of the temporal lobe was used as the lateral border of amygdala.

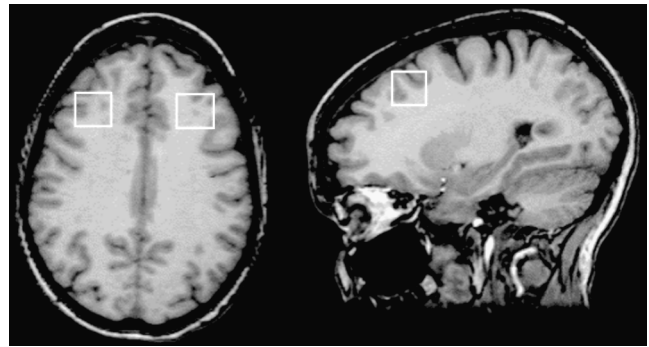


FIG. 2. Placement of magnetic resonance spectroscopy (MRS) voxels in bilateral dorsolateral prefrontal cortex (DLPFC).

rebrum and visually maintaining approximately equal parts gray and white matter (Fig. 2). An investigator blind to diagnosis inspected each voxel placement visually to ensure proper placement fully within the brain and that spectra contain no sizable lipid peaks or rolling baselines. MRS data were acquired using a preselected region of interest for point-resolved spectroscopy (PRESS) with a TR/TE of 2000/35 msec. MRS scans used 32 averages, 1-kHz spectral bandwidth, 1 k data points, and unsuppressed water collected for all spectra. The MRS scan was 1 minute and 44 seconds in length. We were able to obtain an adequate signal-to-noise ratio with this relatively short acquisition time due to the relatively large field strength of 3T. The fully automated PROBE/SV quantification tool (General Electric Medical System, Milwaukee, WI) was used to process MRS data. Each of the five spectral peaks associated with NAA, creatine-phosphocreatine (Cr), choline (Cho), mI, and H_2O was quantified by Levenberg–Marquardt curve fitting over that line region using the standard data processing package by GE mentioned above.

Differences in NAA/Cr ratios from baseline to end of treatment were considered primary outcome measures. Secondary, exploratory analyses of additional metabolite ratios (mI, Cho) were also conducted. Paired t -tests were used to compare pre- and post-valproate ratios. We used Bonferroni correction to account for left and right hemispheric data, and α was set at 0.025 for our main outcome variable, NAA/Cr. We did not correct for exploratory comparisons of mI/Cr and Cho/Cr.

fMRI task

Negative (e.g., a mutilated dog), positive (e.g., puppies), and neutral (e.g., a plate) pictures that were deemed acceptable to a pediatric population were selected from the International Affective Picture System (IAPS) (Lang et al. 1997). The three types of stimuli were organized in blocks, each with six stimuli, with each stimulus presented for 4500 msec with a 500-msec interstimulus interval. Subjects were asked to indicate how each picture made them feel by pressing one of three buttons corresponding to “negatively,” “neutrally,” and “positively.” Stimuli were projected onto a screen using a custom-built magnet compatible projection system (Sanyo, San Diego). A custom-built button box was used to record responses.

fMRI data acquisition

Images were acquired on a 3T GE Signa scanner using a standard GE whole head coil. The following spiral pulse sequence parameters were used: TR = 2000 msec, TE = 30 msec, flip angle = 80°, field of view (FOV) = 200, 28 slices, 64×64 matrix, and 1 interleave. To reduce field inhomogeneities, an automated high-order shimming method based on spiral acquisitions was used before acquiring functional MRI scans (Kim et al. 2000). To aid in localization of the functional data, high-resolution T1 weighted spoiled gradient recalled (SPGR) 3D MRI sequences with the following parameters used: TR = 35 msec, TE = 6 msec, flip angle = 45°, FOV = 24 cm, 124 slices in coronal plane, 256×192 matrix.

Image preprocessing

fMRI data were preprocessed using SPM2 (<http://www.fil.ion.ucl.ac.uk/spm>). Images were reconstructed, corrected for movement, and normalized to Montreal Neurological Institute (MNI) coordinates. Images were then resampled every 2 mm and smoothed with a 4-mm Gaussian kernel. MNI coordinates were transformed to stereotaxic Talairach coordinates using a nonlinear transformation (Brett et al. 2002; <http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html>).

fMRI statistical analysis

Statistical analysis was performed on individual and group data using the general linear model and the theory of Gaussian random fields as implemented in SPM2 (Wellcome Department of Cognitive Neurology, London, UK). Each subject's data were globally scaled, high passed filtered at 120 seconds, and analyzed using a balanced design with

models that computed contrast images of negative minus neutral conditions. These models also included additional contrast images computing repeated measures activation differences between the subject's baseline scan and week 12 scan for the negative-neutral contrasts described above. Resultant contrast images were analyzed using a general linear model to determine voxel-wise *t*-statistics.

fMRI regions of interest analysis

Our hypotheses of the role of the DLPFC and amygdala in BD were tested by measuring activation in these regions using spherical regions of interest (ROIs) (5 mm radius). Both right (22, -2, -20) and left (-22, -2, -20) amygdala ROIs were visually placed by a trained research assistant on a group-averaged SPGR scan and examined by 2 trained neuroscientists to verify accuracy of placement. Placement of right (48, 16, 22) and left (-48, 12, 28) DLPFC ROIs were based on prior loci of activation, Brodmann areas 9/45, from a previous study in which pediatric subjects with BD demonstrated greater activation compared to healthy controls when performing the IAPS task, and negative minus neutral pictures contrast (Chang et al. 2004) (Fig. 3).

Activation in the ROIs was quantified as the percentage of voxels within the ROI that surpassed a specified statistical threshold ($Z > 1.67$; $p < 0.05$). Activation differences in each ROI were extracted to a spreadsheet for statistical comparison with clinical scores.

General statistical analyses

Statistical analyses were completed using SPSS 12.0 (<http://www.spss.com/>). Independent *t*-tests were used in comparisons between subsyndromal subjects and healthy con-

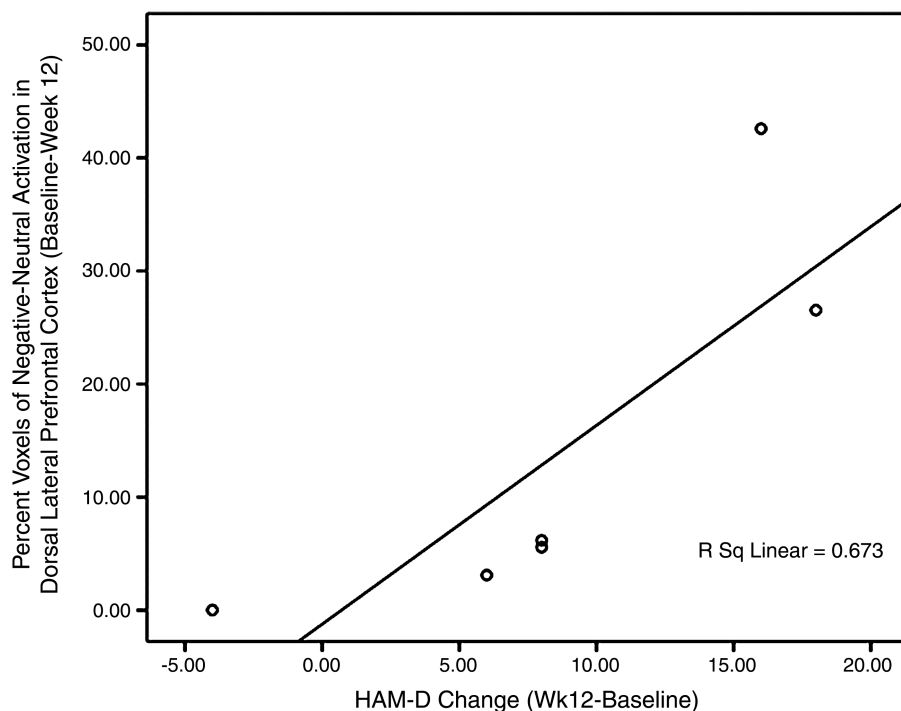


FIG. 3. Change in dorsolateral prefrontal cortex (DLPFC) activation versus change in Hamilton Rating Score for Depression (HAM-D) score in subsyndromal bipolar disease (BD) subjects.

trols for demographic variables, total brain volume (TBV), and ROI activation differences. Repeated measures analysis was used to investigate time point associations within behavioral ratings, ROI activation differences, and clinical scores.

Results

Morphometric data were obtained and usable for all 11 subjects. One subject did not have follow-up MRS data and was excluded from the MRS analysis. For the fMRI analysis, 4 subsyndromal subjects did not have both baseline and 12-week follow-up scans and were excluded. Additionally, 1 subsyndromal subject and 1 healthy control were excluded due to excessive (greater than 10% of the task) combined translational and rotational movement more than 3 mm compared to the first scan of the series. Demographic data are given in Table 1.

Morphometric results

There were no significant changes in TBV in subjects treated with divalproex over 12 weeks ($1549.50 \pm 181.61 \text{ cm}^3$ at baseline versus $1545.52 \pm 186.92 \text{ cm}^3$ after 12 weeks, $p = 0.97$). Healthy controls also did not have changes in TBV over 12

weeks ($1501.28 \pm 232.18 \text{ cm}^3$ at baseline versus $1507.85 \pm 236.75 \text{ cm}^3$ after 12 weeks, $p = 0.96$).

Total amygdala volume in subsyndromal BD subjects did not change significantly over the 12 weeks of divalproex treatment ($3.70 \pm 0.45 \text{ cm}^3$ at baseline versus $3.74 \pm 0.48 \text{ cm}^3$ after 12 weeks, $p = 0.86$; Cohen $d = 0.08$). Furthermore, no difference was found in amygdalar grey matter volume ($3.11 \pm 0.21 \text{ cm}^3$ at baseline versus $3.29 \pm 0.37 \text{ cm}^3$ after 12 weeks, $p = 0.34$).

The amygdala volume in the control group also remained similar over the course of 12 weeks ($3.79 \pm 0.84 \text{ cm}^3$ at baseline versus $4.03 \pm 0.57 \text{ cm}^3$ after 12 weeks, $p = 0.48$; Cohen $d = 0.33$), as did the amygdala grey matter volume ($3.47 \pm 0.74 \text{ cm}^3$ at baseline versus $3.60 \pm 0.56 \text{ cm}^3$ after 12 weeks, $p = 0.63$).

MRS results

There were no significant differences in percent gray and white matter in MRS voxels from baseline compared with week 12. There were no significant differences in pre- or post-divalproex NAA/Cr ratios (see Table 2). The Cohen d was 0.12 for the left and 0.94 for the right, indicating a large effect size for a decrease in right DLPFC NAA/Cr.

TABLE 1. DEMOGRAPHICS OF SUBJECTS

| Measure | Subjects (MRI) | Subjects (MRS) | Subjects (fMRI) | Controls (fMRI) |
|--|--------------------------|---------------------------|-----------------------|----------------------|
| Number of subjects | 11 | 10 | 6 | 5 |
| Mean age (SD) | 11.3 (3.4) | 11.3 (3.6) | 12.1 (4.4) | 14.1 (1.9) |
| Gender | 7 Males; 4 Females | 7 males; 3 Females | 4 Males; 2 Females | 4 Males; 1 Female |
| Ethnicity | | | | |
| Caucasian | 9 | 8 | 5 | 3 |
| Hispanic | 0 | 0 | 0 | 1 |
| Multiracial | 2 | 2 | 1 | 1 |
| IQ (SD) | 112 (13) | 113 (14) | 116 (12) | 116 (16) |
| Handedness | 8 R/1L (2 not specified) | 8 R/0 L (2 not specified) | 6 R/0 L | 5 R/0 L |
| Diagnoses (%) | | | | |
| ADHD | 9 (82) | 8 (80) | 4 (67) | - |
| Anxiety | 5 (45) | 4 (40) | 1 (17) | - |
| Cyclothymia | 2 (18) | 2 (20) | 1 (17) | - |
| Depression | 6 (55) | 5 (50) | 4 (67) | - |
| ODD | 5 (45) | 4 (40) | 1 (17) | - |
| Mean valproate serum level ($\mu\text{g/mL}$) | 82.1 | 81.9 | 78.4 | - |
| Mean decrease in YMRS score over 12 weeks of study | 7.5 | 7.9 | 6.8 | - |
| Mean decrease in HAM-D score | 7.2 | 5.9 | 8.7 | - |
| Past medication exposure (%) | | | | |
| Lithium | 1 (9) | 0 | 0 | - |
| Anticonvulsants | 0 | 0 | 0 | - |
| Antidepressants | 1 (9) | 1 (10) | 1 (17) | - |
| Antipsychotics | 0 | 0 | 0 | - |
| Stimulants | 2 (18) | 2 (18) | 1 (17) | - |

Abbreviations: MRI, Magnetic resonance imaging; MRS, magnetic resonance spectroscopy; fMRI, functional MRI; SD, standard deviation; ADHD, attention-deficit/hyperactivity disorder; ODD, opposition defiant disorder; YMRS, Young Mania Rating Scale; HAM-D, Hamilton Rating Score for Depression.

TABLE 2. ¹H-MRS RESULTS, PRE- AND POSTTREATMENT WITH DIVALPROEX

| | Pre | Post | P |
|-------------|-------------|-------------|------|
| Left DLPFC | | | |
| NAA/Cr | 1.61 ± 0.07 | 1.62 ± 0.10 | 0.88 |
| Cho/Cr | 0.80 ± 0.07 | 0.78 ± 0.26 | 0.68 |
| mI | 0.49 ± 0.05 | 0.50 ± 0.05 | 0.68 |
| Right DLPFC | | | |
| NAA/Cr | 1.69 ± 0.09 | 1.61 ± 0.08 | 0.13 |
| Cho/Cr | 0.82 ± 0.09 | 0.82 ± 0.05 | 0.80 |
| mI/Cr | 0.47 ± 0.05 | 0.46 ± 0.05 | 0.92 |

DLPFC = dorsolateral prefrontal cortex; NAA = *N*-acetylaspartate; Cr = creatine-phospho-creatine; Cho = choline; mI = myo-inositol.

In addition, we performed exploratory analyses on mI/Cr and Cho/Cr ratios and no significant change in these ratios were found. A representative spectrum from one subject is shown in Fig. 4.

fMRI behavioral results

Each individual's ratings were averaged across pictures of the same valence, (negative, neutral, or positive), as classified by the IAPS, to give a subject's mean rating for each valence of the pictures. As expected, there was a significant effect of valence, indicating that all subjects rated the positive, negative, and neutral pictures significantly differently (baseline, $F = 44.73$, $p < 0.001$; week 12, $F = 99.70$, $p < 0.001$). However, repeated measures analysis indicated a significant interaction (Huynh-Feldt, $F = 7.08$, $p = 0.011$) for week-12 behavioral valence scores between subsyndromal subjects and healthy

controls. At week 12, prodromal subjects had significantly less extreme valence ratings for both negative ($t = -2.55$, $p = 0.031$) and positive ($t = 2.31$, $p = 0.046$) valences relative to healthy controls. No significant valence rating differences were found between groups for negative and positive valences at baseline or with neutral valences at either time point.

fMRI brain activation results

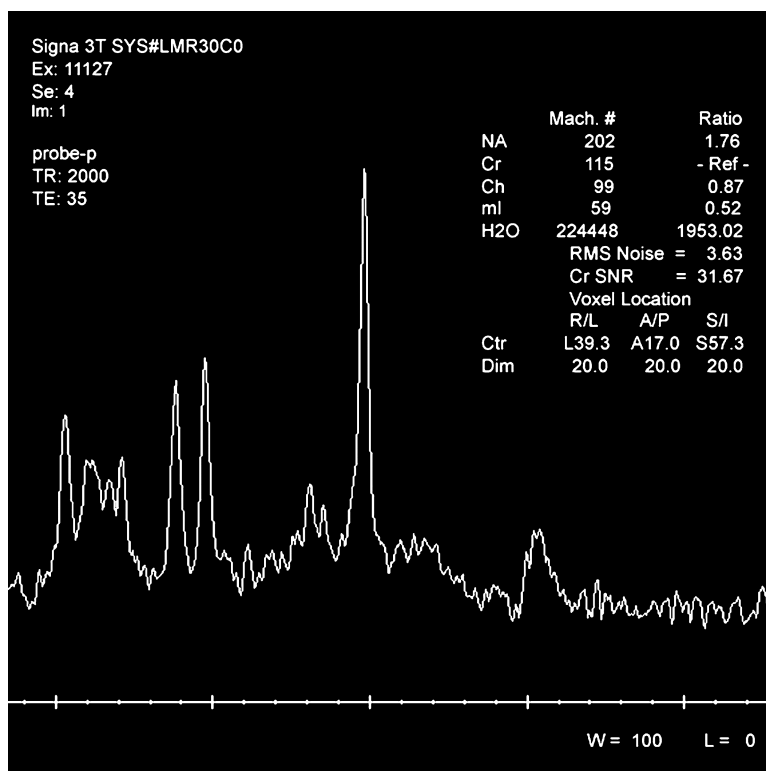
There were no significant differences between subsyndromal and control subjects when comparing activation in the DLPFC or amygdala at baseline (respectively, $t = -0.54$, $p = 0.78$; $t = 0.49$, $p = 0.15$) or at week 12 (respectively, $t = -0.28$, $p = 0.60$; $t = 1.56$, $p = 0.14$). Similarly, there were no significant changes in DLPFC or amygdala activation between baseline and week 12 within the subsyndromal group (respectively, $F = 0.064$, $p = 0.81$; $F = 0.066$, $p = 0.81$) or within the control group (respectively, $F = 0.032$, $p = 0.87$; $F = 0.67$, $p = 0.46$).

Repeated measures analysis resulted in a significant interaction between change in Hamilton Rating Score for Depression (HAM-D) scores and DLPFC activations during baseline scans compared to week 12 scans ($F = 8.218$, $r^2 = 0.673$, $p = 0.046$; Fig. 3). This indicates that greater differential in DLPFC activation from baseline to week 12 was associated with greater improvement in HAM-D score at week 12.

Discussion

We found no significant changes in total brain gray matter volume, amygdalar volume, prefrontal NAA/Cr ratios, or prefrontal amygdalar activation after 12 weeks of divalproex monotherapy in bipolar offspring with subsyndromal mood and behavioral disorders. Despite increasing power by repeated measures analyses, this study was hampered by small sample size and should thus be considered as preliminary

FIG. 4. Representative magnetic resonance spectroscopy (MRS) spectra from 1 subject.



and pilot data. However, effects sizes for morphometric and neurochemical change were generally small, decreasing the possibility of Type II error. The only large effect size found was for a decrease in right DLPFC NAA/Cr in subjects treated with divalproex.

These results are slightly surprising given the preclinical evidence for the neuroprotective qualities of valproate. In animal studies, valproate has been shown to increase levels of the neuroprotective protein bcl-2 in the frontal cortex (Chen et al. 1999; Manji et al. 2000) and activate protein kinases that mediate the effects of neurotrophic factors to stimulate neural dendritic growth (Manji and Lenox 1999). Both lithium and valproate have been found to have neurogenic effects in rat brains and neural stem cells (Hashimoto et al. 2003; Laeng et al. 2004). However, there is little human data in this regard. To our knowledge, there have been no prospective studies of human brain morphometric change following valproate administration. Because of our finding that children with BD and a history of lithium and/or valproate exposure had amygdalar volumes more similar to healthy controls than those children with BD without such exposure, who had decreased volumes (Chang et al. 2005), we had hypothesized that divalproex treatment would result in increased amygdala volume in our subjects. Thus, it is possible that lithium may have more of this effect than valproate. Regarding neurochemistry, two MRS studies of adults with BD failed to find significant effects of valproate on brain NAA, mI, or Glx (Silverstone et al. 2003; Friedman et al. 2004), although neither of these studies was prospective. Similarly, we failed to find significant changes in NAA, mI, and Cho to Cr ratios.

There are even fewer data regarding the effects of valproate on human brain function. In a study of healthy volunteer adults, overall brain activation was increased after 14 days of valproate administration (Bell et al. 2005). Previously, we found that lamotrigine, also an anticonvulsant, led to decreases in amygdalar activation in adolescents with bipolar depression (Chang et al. 2008). Thus, we expected to find similar results in children at-risk for BD treated with another anticonvulsant, divalproex. Again, we did not prove this primary hypothesis.

Our results suggest that behavioral improvement in our subjects may not have been due directly to measurable changes in gray matter, throughout the brain, and in the amygdala specifically. Furthermore, it is also possible that valproate itself simply does not affect these variables. It is also possible that our subjects did not achieve a high enough brain level of valproate to induce measurable change. The achieved mean serum level of 82 $\mu\text{g}/\text{mL}$ is in the suggested therapeutic range for treating adults with BD (Bowden et al. 1994), but toward the lower end of the range suggested for children (80–120 $\mu\text{g}/\text{mL}$) (Kowatch et al. 2005). It is not known if, similar to lithium (Moore 2002), children have lower brain-to-serum ratios of valproate levels than adults due to neurophysiological differences. Children may also require longer treatment than 12 weeks to demonstrate change that was detectable by our methods. A large 4-week trial of extended-release divalproex did fail to demonstrate efficacy over placebo in treating children with acute mania (Wagner et al. in press). However, our subjects showed positive responses in mood symptom severity reduction over 12 weeks, and thus one could reasonably expect corresponding neurobiological change by at least 12 weeks in our subjects.

However, we did, have two interesting findings from the fMRI study. First, subsyndromal BD subjects differed from controls in their ratings of emotionally valenced pictures only after treatment with divalproex. It appeared that their week-12 ratings of negatively valenced pictures were rated less negatively and positive pictures less positively compared with ratings from healthy controls. Subjects may have been desensitized to the pictures because they were shown the same set 12 weeks prior; however, one would expect any such desensitization to be similarly present in healthy controls. Thus, it is possible that treatment with divalproex may have narrowed the subjects' subjective experience of both negativity and positivity. Given the small sample size, this is a highly preliminary finding.

Second, the degree of prefrontal activation decrease was correlated with improvement in depressive symptoms. This finding might indicate why we did not find differences at baseline and at week 12 between subsyndromal subjects and controls in amygdalar or DLPFC activation. There appeared to be a range of both activation and behavioral response, leading to heterogeneity in the sample that may have "washed out" any findings. Correlations with such variables as mood state and response, as done here, may be one solution to addressing this heterogeneity. Furthermore, this finding might indicate that prefrontal structures may be less needed to regulate emotional response after successful treatment with divalproex. It is possible that subjects with greater improvement in depression no longer needed to recruit prefrontal areas to aid in modulating signals from hyperactive subcortical limbic areas. Thus, in this model, DLPFC activation would reflect degree of subcortical limbic activity. Therefore, divalproex may work directly not on prefrontal areas, but potentially in subcortical limbic areas, such as the amygdala. Indeed, one important action of divalproex is potentiation of γ -aminobutyric acid (GABA) neurotransmission (Loscher 2002), and the basolateral nucleus of the amygdala (BLA) is significantly inhibited by GABA-ergic interneurons (Rainnie et al. 1991). Furthermore, electrical kindling of rat amygdala results in decreases of such inhibitory GABA-ergic neurons in the BLA (Callahan et al. 1991; Lehmann et al. 1998). Similar models have been proposed to occur in BD, so that the amygdala may have an increased flow of excitatory activity due to deficiencies in GABA-ergic inhibition (Benes and Berretta 2001). However, it is still possible that divalproex directly affects prefrontal regions as well, leading to decreased activation, but one would then expect less regulatory control over limbic activation, leading to worsening of mood, not improvement.

As mentioned, this study is limited by sample size and thus these results should be taken as preliminary. Our subjects, although all bipolar offspring, also presented with a variety of psychiatric disorders, including ADHD, depression, anxiety, and cyclothymia. This heterogeneity may have led to varying neurobiological responses to valproate and thus our negative MRI findings. A few of our subjects were also previously exposed to psychotropic medications, such as stimulants and antidepressants, which have effects on brain structure and function. For example, increased exposure to antidepressants may lead to decreased amygdalar volume in adolescents with BD (DeBello et al. 2004). We used ratios of NAA to Cr-Pcr and did not obtain absolute concentrations of NAA due to methodological issues. Specifically, p files for spectra were not saved correctly, so that later analysis with programs to

calculate absolute concentrations, such as the LC Model, was not possible. Thus, changes in Cr over time may have obscured actual changes in NAA concentrations. Finally, there may have been changes in other regions of the brain that we did not study, such as hippocampus, ventrolateral prefrontal cortex (PFC), or anterior cingulate, where others have found neurometabolite change in response to psychotropic medications (DelBello et al. 2006; Patel et al. 2008).

Nonetheless, this is the first study to investigate the neurobiological effects of valproate in a pediatric population, and a population at genetic risk for BD. Our results may indicate that behavioral change may predate neurobiological change that was detectable by our methods. Clearly, prospective neuroimaging studies with larger samples of children with mood disorders treated with psychotropic agents over longer periods are needed to clarify the neurobiological effects of these medications.

Disclosures

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