◆ Human Brain Mapping 33:501–510 (2012) ◆

Hippocampal Morphology in Lithium and Non-Lithium-Treated Bipolar I Disorder Patients, Non-Bipolar Co-Twins, and Control Twins

Theo G.M. van Erp,[1]* Paul M. Thompson,[2] Tuula Kieseppä,[3,4] Carrie E. Bearden,[5,6] Alexandria C. Marino,[7] Gil D. Hoftman,[7] Jari Haukka,[3] Timo Partonen,[3] Matti Huttunen,[3] Jaakko Kaprio,[3,8,9] Jouko Lönnqvist,[3] Veli-Pekka Poutanen,[10] Arthur W. Toga,[2] and Tyrone D. Cannon[5,6]

[1]*Department of Psychiatry and Human Behavior, UCI, Irvine, California*
[2]*Laboratory of Neuro Imaging, Department of Neurology, UCLA, Los Angeles, California*
[3]*Department of Mental Health and Alcohol Research, National Institute for Health and Welfar, Helsinki, Finland*
[4]*Department of Psychiatry, Univ. of Helsinki Central Hospital, Helsinki, Finland*
[5]*Department of Psychology, UCLA, Los Angeles, California*
[6]*Department of Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, California*
[7]*Department of Psychology, UCLA, Los Angeles, California*
[8]*Department of Public Health, University of Helsinki, Helsinki, Finland*
[9]*Institute of Molecular Medicine, University of Helsinki, Helsinki, Finland*
[10]*Department of Radiology, University of Helsinki Central Hospital, Meilahti Clinics, Helsinki, Finland*

Abstract: *Background*: Bipolar I disorder is a highly heritable psychiatric illness with undetermined predisposing genetic and environmental risk factors. We examined familial contributions to hippocampal morphology in bipolar disorder, using a population-based twin cohort design. *Methods*: We acquired high-resolution brain MRI scans from 18 adult patients with bipolar I disorder [BPI; mean age 45.6 ± 8.69 (SD); 10 lithium-treated], 14 non-bipolar co-twins, and 32 demographically matched healthy comparison twins. We used three-dimensional radial distance mapping techniques to visualize hippocampal shape differences between groups. *Results*: Lithium-treated BPI patients had significantly larger global hippocampal volume compared to both healthy controls (9%) and non-bipolar co-twins (12%), and trend-level larger volumes relative to non-lithium-treated BPI patients (8%). In contrast, hippocampal volumes in non-lithium-treated BPI patients did not differ from those of non-bipolar co-twins and control twins. 3D surface maps revealed thicker hippocampi in lithium-treated BPI probands compared with control twins across the entire anterior-to-posterior extent of the *cornu ammonis* (CA1 and 2) regions, and the anterior part of the subiculum. Unexpectedly, co-twins also showed significantly thicker hippocampi compared with control twins in regions that partially overlapped those showing

Contract grant sponsor: NIHM; Contract grant numbers: MH52857, K23MH74644, MH48207, MH43880, and 1; Contract grant sponsor: NIH; Contract grant number: U54 GM072978-01.

*Correspondence to: Theo G.M. van Erp, Department of Psychiatry and Human Behavior, School of Medicine, University of California, Irvine, 5251 California Avenue, Suite 240C, Irvine, California 92617. E-mail: tvanerp@uci.edu

Received for publication 18 March 2009; Revised 16 November 2010; Accepted 17 November 2010

DOI: 10.1002/hbm.21239

Published online 31 March 2011 in Wiley Online Library (wileyonlinelibrary.com).

effects in the lithium treated BPI probands. *Conclusions*: These findings suggest that regionally thickened hippocampi in bipolar I disorder may be partly due to familial factors and partly due to lithium-induced neurotrophy, neurogenesis, or neuroprotection. Unlike schizophrenia, hippocampal alterations in co-twins of bipolar I disorder probands are likely to manifest as subtle volume excess rather than deficit, perhaps indicating protective rather than risk effects. *Hum Brain Mapp 33:501–510, 2012*.

Key words: bipolar disorder; magnetic resonance imaging; hippocampus; shape; volume; mood disorders; twin; morphology

INTRODUCTION

Bipolar I is a highly heritable psychiatric disorder, with estimates of additive genetic contributions to disease liability in the range of 69–100% [Kieseppa et al., 2004]; see also [Bertelsen et al., 1977; Cardno et al., 1999; Kendler et al., 1993; McGuffin et al., 2003]. Nevertheless, the predisposing genes and their mechanisms of action are yet to be determined. One approach to search for susceptibility genes for complex disorders is to use endophenotypic markers, or measures thought to be closer to a disorder's pathophysiology than clinical diagnoses [Gottesman and Gould, 2003]. Declarative memory impairments, that may involve hippocampal pathology, have been observed in patients with bipolar disorder [Frey et al., 2007] and their unaffected relatives [Gourovitch et al., 1999; Kieseppa et al., 2005]. In this study, we therefore examined hippocampal morphology in twin pairs discordant for bipolar I disorder, using surface-based anatomical modeling methods [Thompson et al., 2004] to determine the extent to which hippocampal morphological abnormalities in bipolar I disorder are associated with predisposing familial or treatment-related factors.

Hippocampal volumes in adult and pediatric patients with bipolar disorder have either been shown to be enlarged [Beyer et al., 2004; Kemmerer et al., 1994], reduced [Blumberg et al., 2003; Frazier et al., 2005; Rossi et al., 1991] or equivalent [Altshuler et al., 2000; Brambilla et al., 2003; Hauser et al., 2000; Pearlson et al., 1997; Sax et al., 1999; Strakowski et al., 1999, 2002; Strasser et al., 2005] to those of a variety of control groups. These discrepancies may be in part due to differences in clinical characteristics such as duration and severity of illness, age, number of prior episodes, and medication use. More recently, consistent with the lithium-induced neurotrophy, neuroprotection, or neurogenesis [Chen et al., 2000; Manji et al., 2000], several studies reported larger hippocampal volumes in lithium-treated patients with bipolar disorder [Bearden et al., 2007b; Germana et al., 2010; Yucel et al., 2007, 2008] but see some conflicting findings [Javadapour et al., 2010; Rimol et al., 2010]. In addition, unmedicated bipolar patients may show localized hippocampal reductions compared with healthy controls [Bearden et al., 2007b], effects that may not be observed in small samples when confounding medication effects are not to some extent taken into account [Mamah et al., 2010]. These findings suggest that bipolar illness may be associated with subtle hippocampal volume deficits, which may be attenuated or reversed by lithium treatment.

Very few neuroanatomic studies of bipolar disorder have examined those at genetic high risk for the illness. Apart from the small twin study by Noga et al. [2001], in which an absence of typical asymmetry of the hippocampi was observed among six healthy co-twins of six bipolar probands when compared with 12 control twins, to our knowledge, none of the bipolar disorder studies that included relatives [Ahearn et al., 1998, 2002; Gulseren et al. 2006; Hajek et al., 2008a,b; 2009; Kieseppa et al., 2003; McDonald et al., 2006; McIntosh et al., 2006; Noga et al., 2001; van der Schot et al., 2009] have examined hippocampal morphology (for review see Hajek et al. [2005]). Here we set out to create the first detailed hippocampal surface maps comparing patients with bipolar I disorder and non-bipolar co-twins with control twins, using a highly sensitive method that creates group average maps of three-dimensional (3D) hippocampal surface models, thus providing detailed maps of local hippocampal thickness measurements [Thompson et al., 2004]. Hippocampal surface anatomy was carefully matched across individuals to provide accurate and spatially refined localizations of group differences.

Based on findings to date, we hypothesized that: (1) non-lithium-treated bipolar I patients and non-bipolar co-twins of BPI patients would show hippocampal volume deficits relative to healthy control twins, and (2) that lithium-treated bipolar I patients would have larger hippocampi than non-lithium-treated patients, non-bipolar co-twins, and healthy control twins.

METHODS

Subjects

The National Hospital Discharge Register of Finland was searched for patients with ICD-8 [WHO, 1967] codes of 296.10 or 296.30, or a DSM-III-R codes of 296.4, 296.5, or 296.6 during 1969 to 1991. Subsequently, the National Population Register and the Finnish Twin Cohorts [Kaprio et al., 1990] were queried to locate twins born between 1940 and 1969. This comprehensive search identified 59

TABLE I. Sample demographics

	Lithium-treated BPI Probands ($N = 10$)	BPI probands no lithium ($N = 8$)	Co-twins ($N = 14$)	Control twins ($n = 32$)	P-value
Age in years (SD)	45.60 (8.69)	42.50 (6.76)	44.64 (7.38)	47.19 (3.86)	0.21
Sex (Female)[a]	7 (70)	3 (38)	9 (64)	15 (47)	0.37
Education (SD)[b]	4.30 (2.36)	3.63 (2.97)	3.00 (2.04)	3.69 (1.09)	0.40
Monozygotic[a]	2 (20)	2 (25)	1 (7)	6 (19)	0.57
Right handed[a]	10 (100.00)	8 (100)	14 (100)	31 (96.88)	1.00
Life-time alcohol dependence[a]	1 (10)	4 (50)	1 (7)	2 (6)	0.02
Current alcohol dependence[a]	1 (10)	0 (0)	0 (0)	1 (3)	0.53
Lifetime anxiety disorder[a]	3 (30.00)	4 (50)	1 (7)	2 (6)	0.009
Lithium (mg/day)[c]	900 (600–1,200)	0	0	0	
Any psychotropic medication[a]	9 (90)	3 (38)	0	0	
Length of use of medication (years)[c]	14.3 (4.5–26)	8.3 (0.3–27)	0	0	

[a]Data represented as n (%).
[b]Education classified according the Structural Clinical Interview for DSM-IV.
[c]Data represented as mean (range).

twins, who were invited to participate in the study with their co-twin [Kieseppa et al., 2000].

All participants were assessed with the Structured Clinical Interview for DSM-IV Disorders (SCID [Spitzer et al., 1997]), as detailed elsewhere [Cannon et al., 2000; Kieseppa et al., 2000, 2004]. Zygosity was determined based on genetic marker analysis in all control and index pairs [Cannon et al., 2002].

Because our collection method was population-based, we included all valid cases, including individual twin subjects. The numbers of bipolar twins and co-twins were relatively small, and we wanted to maximize the utility of the information available to us. Thus, after diagnostic ascertainment, 18 BPI probands (6 without a co-twin; 10 Lithium-treated) and 14 co-twins (6 without a proband) were selected for imaging. Only four of the bipolar twins were from monozygotic pairs. In cases for which the co-twin did not have a proband that was included in the study, proband diagnosis of BPI was based on all information obtained from medical records, forensic reports, and interviews with health personnel and the co-twin. Two twin pairs were concordant for BPI. One index pair was opposite sex (male/female). Exclusion criteria for all groups were any other psychotic or mood disorders, neurological disorders affecting the brain, brain injuries, or current substance abuse. Nine out of ten lithium-treated patients and three out of eight non-lithium-treated patients received antidepressant medication. None of the bipolar patients was treated with valproate. Five out of the eight non-lithium-treated patients received no medication (see Tables I and II for sample details). Three of the patients in the non-lithium group in our sample had a prior history of lithium use 20 years before they participated in this study (1 for 2 months, and the others for 2 years). None of the subjects had lithium toxicity events within the 12 months prior to the time of image acquisition.

A control group of 32 twins from 32 healthy twin pairs without any psychotic or mood disorder, of which six from MZ pairs, was recruited from the same Finnish twin cohort and matched to the mean age [$F(2,61) = 1.72$, $P = 0.19$], sex (χ^2_2 1.24, P 0.54) and zygosity distributions (χ^2_2 1.37, P 0.57) of the patient and co-twin samples. The groups showed a trend-level difference in the frequency of lifetime alcohol abuse (Fisher's Exact Test; χ^2_2 5.35, P 0.08) and significant differences in the frequency of lifetime anxiety disorder diagnoses (Fisher's Exact Test; χ^2_2 10.29, P 0.009), both with higher prevalence in the bipolar disorder patients.

The study was approved by the Ministry of Social Affairs and Health in Finland, the Ethics Committee of the National Public Health Institute of Finland, and the

TABLE II. Twin sample by group and zygosity

Diagnostic status of twin pair (number of pairs)	Zygosity	N pairs	N participants
MRI data available for both twins			
BPI Li+/BPI Li−	MZ	1	2
BPI Li+/BPI Li−	DZ	1	2
BPI Li+/Healthy	MZ	1	2
BPI Li+/Healthy	DZ	4	8
BPI Li−/Healthy	DZ	3	6
MRI data only for probands			
BPI Li+/(Healthy)	DZ	3	3
BPI Li−/(Healthy)	MZ	1	1
BPI Li−/(Healthy)	DZ	2	2
MRI data only for co-twins			
(BPI)/Healthy	DZ	6	6
Healthy/(Healthy)	MZ	6	6
Healthy/(Healthy)	DZ	26	26
Total		54	64

BPI Li+, Bipolar I patient on Lithium; BPI Li−, Bipolar I patient not on lithium.

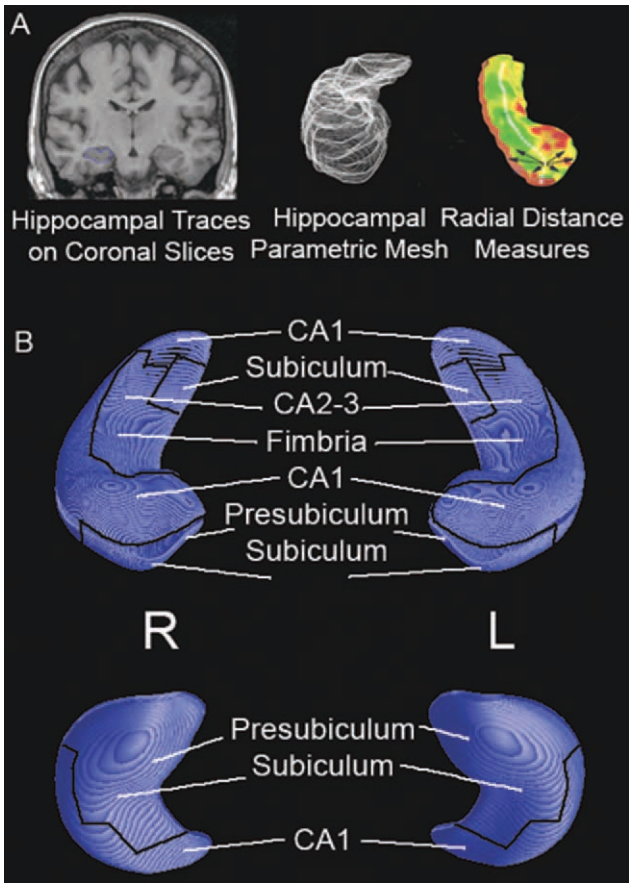

Figure 1.
3D Representation of Hippocampal Cytoarchitectonic Subregions. Figure adapted from Frisoni et al., Neuroimage 2006, 32, 104-110. Traces outlining the hippocampus—including the cornu ammonis (CA) regions 1–4, dentate gyrus (DG), and subiculum—were created with MultiTracer (http://air.bmap.ucla.edu/MultiTracer/) on coronal slices using a protocol described previously (Thompson et al., 2004). Subsequently, anatomical mesh modeling methods (Thompson et al., 1996) were used to match equivalent hippocampal surface points across subjects and groups. Briefly, top and bottom hippocampal surfaces were digitized to create parametric meshes of 150 × 100 surface points. This procedure allows for measurements to be made at corresponding 3D surface locations in each subject that may then be compared statistically. A 3D medial space curve through the centroid of the hippocampal cross-sections was computed, and at each surface point the radial distance between the surface point and the center was computed (**a**) allowing detailed mapping of hippocampal morphology (**b**). Part A of the figure was adapted from Figure 7 in Thompson et al. British Journal of Radiology (2007) 80, S78-S91.

University of California Los Angeles Institutional Review Board and complied with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written, informed consent was obtained from all participants after they had received a complete description of the study.

Image Acquisition

The scanning protocol was identical to that used in our other twin studies [Cannon et al., 2002; Thompson et al., 2001]. Briefly, T1-weighted MP-RAGE (magnetization prepared rapid gradient echo) scans of the brain (TR/TE = 11.4/4.4 ms, sagittal orientation, matrix = 256 × 256 × 128, FOV = 250 mm, resolution = 0.98 × 0.98 × 1.2) were acquired on a 1.0-Tesla scanner (Siemens, Inselin, NJ) at a private medical center (Teslamed, Helsinki, Finland).

Hippocampal Shape Protocol

Prior to tracing, to correct for differences in image intensity and overall brain size, individual brain images were corrected for radio frequency field non-uniformity using Montreal Neurological Institute's (MNI) N3 [Sled et al., 1998] and aligned to the ICBM-305 average brain template with a least-squares 9-parameter registration model implemented using MNI's "mritotal" [Collins et al., 1994]. Hippocampal measures were taken as outlined in Figure 1. Intra-rater (AM) and inter-rater (AM, GDH) reliabilities as measured by intraclass correlations were high for left and right hippocampal volume (> 0.90; > 0.90), surface area (>0.95; >0.93), and length (>0.92; >0.86). Reliability maps for the radial measures using this method have been reported elsewhere [Thompson et al., 2004].

Statistical Analyses

Group differences in hippocampal morphology were examined using mixed model regression analyses [Proc Mixed, Statistical Analysis Software (SAS)] predicting hippocampal volume, length, surface area, and thickness (i.e., radial distances from the medial core of the hippocampus) from variables including group (lithium-treated BPI patients, BPI not on lithium, co-twin, control), hemisphere (left, right), and a group × hemisphere interaction (Table III). Hemisphere entered the model as a within-subject repeated measure. Non-independence of measures among co-twins was controlled for by including twin pair as a random variable and adjusting the model error degrees of freedom with the Satterthwaite option. Sex, age, lifetime alcohol dependence, and lifetime anxiety disorder entered the model as covariates. Significant volumetric findings are represented in bar graphs and least square mean differences in radial distance. Where the direction of the effect was predicted p-values reported are one-tailed. *P*-values describing the significance of group differences were plotted at each hippocampal surface point using a color code to produce a statistical map. Permutation methods [Bullmore et al., 1999; Thompson et al., 2003] were used to assess the significance of the statistical maps, and to correct for multiple comparisons. In each case, the covariate (group membership) was permuted 100,000 times on an SGI Reality Monster supercomputer with 64 internal R10000 processors, and a null distribution was developed

TABLE III. Absolute hippocampal measures (SD) by group and hemisphere

	BPI lithium ($n = 10$)	BPI No lithium ($n = 8$)	Co-twin ($n = 14$)	Control twin ($n = 32$)
Volume (mL)				
Left	3,828 (221)	3,436 (196)	3,463 (292)	3,534 (396)
Right	3,877 (272)	3,570 (293)	3,549 (294)	3,619 (347)
Area (mm^2)				
Left	1,865 (125)	1,748 (80)	1,744 (110)	1,699 (131)
Right	1,861 (110)	1,771 (116)	1,766 (115)	1,698 (115)
Length (mm)				
Left	36.6 (1.7)	36.0 (2.8)	36.4 (2.0)	36.0 (2.2)
Right	35.3 (2.8)	35.3 (2.3)	35.9 (2.2)	35.7 (1.9)

for the area of the hippocampal surface with group difference statistics above a fixed threshold ($P < 0.05$) in the significance maps. An algorithm was then used to determine the significance probability for the overall difference patterns in each map [Thompson et al., 2003], after the appropriate correction for multiple comparisons.

RESULTS

Hippocampal Volume

Groupwise comparisons of hippocampal volumes were performed to provide a context for the 3D hippocampal maps. There were significant main effects of group (BPI Li+, BPI Li-, co-twin, control) [$F(3,56) = 3.15$, $P = 0.03$] and hemisphere [$F(1,60) = 5.53$, $P = 0.02$] on hippocampal volume. BPI Li+ patients had significantly larger hippocampal volumes than the non-bipolar co-twins ($t_{56} = 2.96$, $P < 0.003$) and control twins ($t_{56} = 2.58$, $P = 0.005$), and trend-level significantly larger hippocampal volumes than the BPI Li- patients ($t_{56} = 1.60$, $P < 0.06$) (Fig. 2). Across groups, the right hippocampus was larger than the left ($t_{60} = 2.35$; $P = 0.02$).

Hippocampal Surface Area

There was a main effect of group [$F(3,55) = 6.79$, $P = 0.0006$] and lifetime alcohol dependence [$F(1,55) = 4.31$, $P = 0.04$] on hippocampal surface area. BPI Li+ patients (LSM = 1,810 mm^2, SE = 38) had significantly larger hippocampal surface areas than control twins ($t_{55} = 4.43$, $P < 0.0001$; LSM = 1,639 mm^2, SE = 35) and non-bipolar co-twins ($t_{55} = 2.79$, $P = 0.007$; LSM = 1,692 mm^2; SE = 38), and non-significantly larger hippocampal areas than the BPI Li- patients ($t_{55} = 1.03$, $P = 0.31$; LSM = 1,756 mm^2, SE = 36). The BPI Li- group had larger hippocampal surface areas than the control group ($t_{55} = 2.27$, $P = 0.03$). Across groups, those with lifetime alcohol dependence (LSM = 1,672 mm^2, SE = 45) had smaller hippocampal areas than those without ($t_{55} = 2.07$, $P = 0.04$; LSM = 1,777 mm^2, SE = 20).

Hippocampal Length

Across groups, left hippocampi were longer than right hippocampi ($t_{60} = 2.76$, $P = 0.008$) and those with a lifetime anxiety disorder had shorter hippocampi than those without ($t_{56} = -2.15$, $P = 0.04$). None of the other predictors showed significant main or interaction effects.

3D Hippocampal Maps

3D hippocampal surface maps showed significantly larger hippocampus thickness in the BPI Li+ patients compared with the control twins (L: $P < 0.04$, R: $P < 0.001$, for BPI Li+ > control, permutation test), and significantly larger right hemisphere hippocampus thickness in BPI Li+ patients compared with the unaffected co-twins (L: $P = 0.31$, R: $P < 0.03$, for BPI Li+ > co-twin, permutation test), across the entire anterior-to-posterior extent of the cornu ammonis (CA 1 and 2) regions, and the anterior part of the subiculum, but no significantly larger hippocampus thickness in the BPI Li+ group compared with the BPI Li-

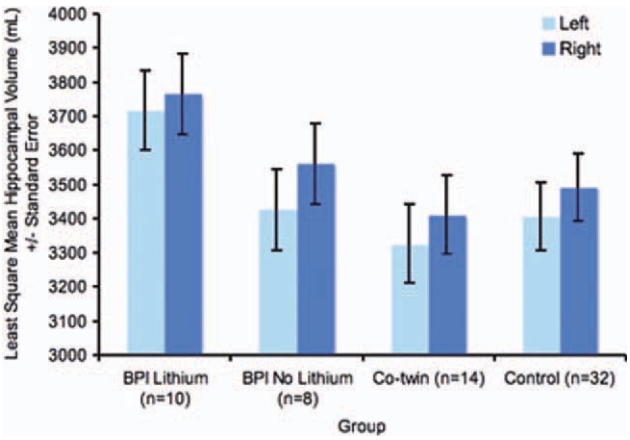

Figure 2.
Hippocampal Volumes in Lithium-Treated Bipolar I Disorder Patients, Non-Lithium-Treated Bipolar I Disorder Patients, Non-Bipolar Co-twins, and Healthy Twins. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

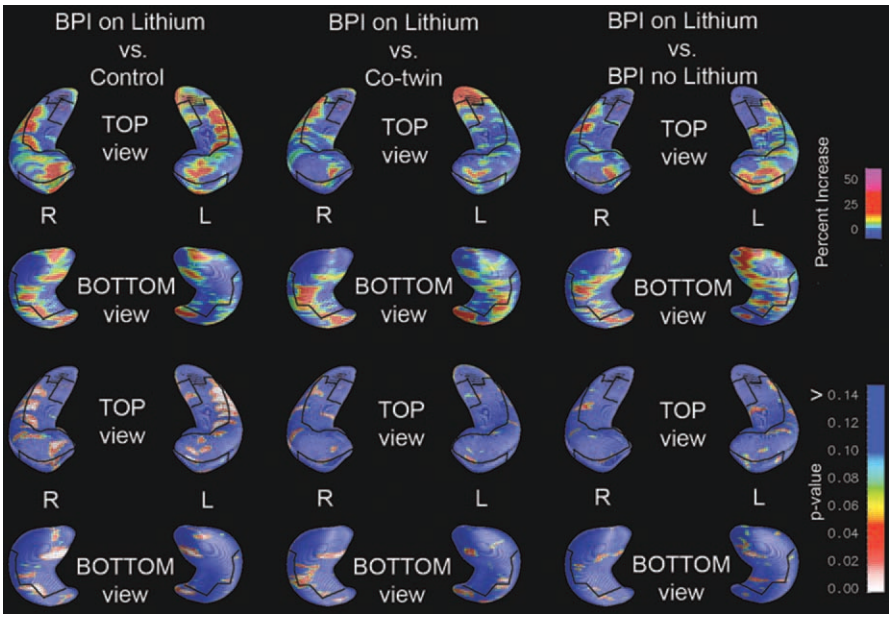

Figure 3.
3D Hippocampal Maps: Lithium-treated bipolar probands vs. control twins (left), vs. non-bipolar co-twins (middle), and vs. non-lithium-treated bipolar probands (right). The top two rows reflect percent gray matter increase and the bottom two rows corresponding *P*-values.

group (L: $P = 0.20$, R: $P = 0.36$, for BPI Li+ > BPI Li-, permutation test) (Fig. 3).

Further map-wise comparisons revealed that the non-bipolar co-twins also had larger hippocampus thickness relative to controls, along the border of the cornu ammonis (CA 1 and 2) region and the anterior subiculum, which were significant on the right side (L: $P = 0.34$; R: $P = 0.01$, for Co-twin > Control, permutation test). To some extent, these areas overlapped with regions showing lithium-associated effects in BPI probands (Fig. 4).

DISCUSSION

The principal findings of this study were as follows: (1) global hippocampal volume was larger in bipolar I patients treated with lithium, relative to age- and sex-matched healthy control twins and non-bipolar co-twins of bipolar probands, and trend-level larger relative to patients not treated with lithium, with the excess most pronounced in the cornu ammonis 1 and 2 and the anterior subiculum hippocampal sub-regions, and (2) despite no differences in global hippocampal volume, significant regional right hippocampal thickening was present in non-bipolar co-twins compared with control twins, and the thicker regions partially overlapped those showing lithium-associated regional thickening in bipolar I probands.

Lithium treatment was associated with significantly larger hippocampal volumes in BPI versus healthy control

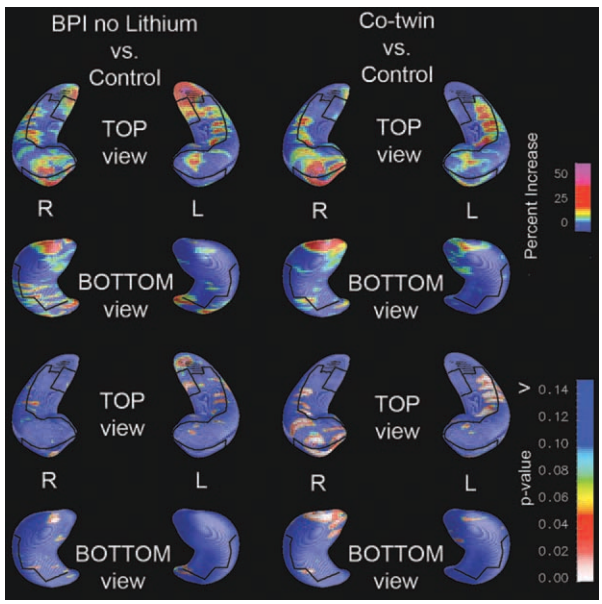

Figure 4.
3D Hippocampal Maps: Non-lithium treated bipolar probands vs. controls (left) and co-twins vs. controls (right). The top two rows reflect percent gray matter increase and the bottom two rows corresponding p-values for the contrasts of non-lithium treated BPI Probands vs. Controls and Co-twins vs. Controls. The maps show a significant increase in right hippocampal volume for co-twins compared with controls, in particular in CA1.

twins (9%) and non-bipolar co-twins (12%), and trend-level larger hippocampal volumes compared with non-lithium-treated BPI probands (8%). These findings corroborate prior observations of larger lithium-associated hippocampal volumes in bipolar patients, in cross-sectional [Bearden et al., 2007b; Beyer et al., 2004; Germana et al., 2010; Yucel et al., 2008] and longitudinal designs [Yucel et al., 2007], and the observation that patient-control group differences are positively associated with the percent of lithium-treated patients across studies [Kempton et al., 2008]. The hippocampus is known to be a brain region in which neurogenesis occurs even in adulthood [van Praag et al., 2002]. It is therefore plausible that the regional hipopcampal thickening in Li+ compared with Li- patients is in part due to lithium-induced neurogenesis. The extent to which the observed hippocampal thickening is due to lithium-induced neurogenesis, neurotropy, or neuroprotection, as reported in preclinical studies [Chen et al., 2000; Frey et al., 2006], warrants investigation in future studies. It must be noted that three of the patients in the non-lithium group in our sample had a prior history of lithium use. However, their exposure was relatively short (1 for 2 months, and the others for 2 years), in each case occurred more than 20 years before they participated in this study, and their mean hippocampal volumes were similar to those not treated with lithium (3,373 mm^3 and 3,519 mm^3 for left and right hippocampus, respectively). No significant associations between the hippocampal morphological measures and lithium dose were observed.

The identification of abnormal hippocampal structure may help to elucidate the underlying pathophysiological mechanisms associated with bipolar illness, and may also indicate functional systems that may be disturbed in the illness. Hippocampal pathology may underlie the declarative memory impairments observed in patients with bipolar disorder [Frey et al., 2007] and their unaffected relatives [Gourovitch et al., 1999; Kieseppa et al., 2005]. In fact, Yucel et al. [2007, 2008] reported an increase in hippocampal volume and verbal declarative memory performance after lithium treatment [Yucel et al., 2007], though practice effects were not controlled for.

As cortical gray matter is larger in lithium-treated patients versus controls [Moore et al., 2000; Sassi et al., 2002], it is unlikely that lithium effects are limited to the hippocampus. Detailed mapping of lithium effects on cortical gray matter in this sample is currently in progress. One such prior study found regional differences in the magnitude of lithium effects, with greatest effects in the bilateral cingulate and paralimbic cortices [Bearden et al., 2007a]. Possible differences in subcortical versus cortical lithium effects warrant further exploration, particularly with regard to their timing and association with treatment response. For instance, lithium-associated gray matter effects may be exacerbated in the hippocampus, as lithium acts on several biochemical pathways that may exert relatively large effects in the hippocampus; for review see [Shaltiel et al., 2007]. More specifically, some of its effects appear to include a reduction of glutamate-induced, NMDA receptor-mediated excitotoxicity [Nonaka and Chuang, 1998] and an increase in the expression of brain-derived neurotrophic factor (BDNF) [Frey et al., 2006; Hashimoto et al., 2002]. While further research is needed, it has been suggested that lithium may also be of use in the treatment of other neuropsychiatric disorders [Chen et al., 2000] and possibly also in neurodegenerative disorders.

Other studies have found larger gray matter volumes in lithium vs. non-lithium-treated bipolar patients, but our finding of regionally larger hippocampal thickness in non-bipolar co-twins relative to controls is novel. This finding clearly warrants replication, though it is consistent with a recent study that reported larger gray matter volume in the left parahippocampal gyrus in healthy offspring from parents diagnosed with bipolar disorder [Ladouceur et al., 2008]. The pattern of larger local hippocampal thickness among the non-lithium treated bipolar I patients was very similar to that of the non-ill co-twins, but was not statistically significant. Although co-twins had never taken lithium or other mood -stabilizing medication, they nevertheless showed thickened hippocampi in similar hippocampal regions as bipolar I patients on lithium therapy - specifically, portions of the cornu ammonis (CA 1 and 2) subfields and the anterior subiculum. The observation that the thickening of the hippocampi in lithium treated patients is in similar areas as the hippocampal thickening in the unaffected and unmedicated co-twins may suggest that lithium acts on similar molecular pathways as familial (genetic or shared environmental) risk or protective factors for mood instability.

Usually mean differences observed in co-twins of patients with psychiatric disorders are interpreted as risk effects. However, given that lithium, used as a first-line treatment for bipolar disorder, is associated with greater hippocampal thickness, it is tempting to speculate that the thicker hippocampi in the co-twins compared with controls protect these co-twins against the pathological fluctuations in mood observed in bipolar I patients, and that lithium-induced hippocampal thickening may lead to similar "protective" effects in patients. Clearly, caution is warranted given the cross-sectional nature of these findings, but the thicker hippocampi in the co-twins of BPI patients relative to the controls is a striking addition to the current findings in the literature and clearly warrants follow-up with larger family and twin samples.

Among the weaknesses of this study are the relatively small sample sizes for each of the groups, and the cross-sectional rather than longitudinal design with regard to the lithium treatment effects. Furthermore, we cannot exclude possible confounding effects of antidepressants. In addition to lithium, 9 of the Li+ and 3 of the Li- patients were treated with anti-depressant medications, which have recently been shown to be associated with larger hippocampal volumes in depressed patients [Malykhin et al., 2010]. Within-subject longitudinal studies are needed to

better disentangle potential effects of both lithium and anti-depressants on regional hippocampal morphology in bipolar disorder patients. Lifetime diagnoses of anxiety disorder and alcohol dependence were present more often bipolar disorder patients compared with controls. Excessive alcohol intake in general [Wilhelm et al., 2008] and anxiety in bipolar disorder [Simeonova et al., 2009] are correlated with reduced hippocampal volume and are therefore not likely to explain the observed effects. Because of the small number of monozygotic twin pairs, we were unable to compare monozygotic vs. dizygotic twin pairs and due to the small number of twin pairs overall we were unable to fully implement matched-pair comparisons, which is a key strength of the twin design. Strengths of the study are that this is the first study to examine regional hippocampal alterations in bipolar probands and non-bipolar co-twins; we included a homogeneous group of probands with bipolar I disorder diagnoses only; and study participants were ascertained from a population-based twin sample, such that the findings can be generalized to all twins in the cohort.

The familial liability for bipolar I disorder, in non-bipolar co-twins, appears to be associated with larger regional hippocampus thickness—larger thickness that is also observed in bipolar I patients treated with lithium. These findings are promising in that they show regional effects on hippocampal morphology, but the study of lithium effects requires pre and posttreatment follow-up. Genetic and environmental influences on hippocampal morphology should also be examined with studies that study sufficient numbers of monozygotic and dizygotic co-twins. Future work should also examine whether lithium treatment modulates limbic-cortical network activity and connectivity [Mayberg, 1997, 2003] using physiological measures such as functional magnetic resonance imaging and electroencephalography both pre and postlithium treatment.

ACKNOWLEDGMENTS

The authors thank Ulla Mustonen for her role in recruitment and clinical evaluation as well as the twins who participated in the study. The Finnish Twin Cohort study forms part of the Academy of Finland Centre of Excellence in Complex Disease Genetics.

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