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Insulin Resistance, Diabetes Mellitus, and Brain Structure in Bipolar Disorders

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Type 2 diabetes mellitus (T2DM) damages the brain, especially the hippocampus, and frequently co-occurs with bipolar disorders (BD). Reduced hippocampal volumes are found only in some studies of BD subjects and may thus be secondary to the presence of certain clinical variables. Studying BD patients with abnormal glucose metabolism could help identify preventable risk factors for hippocampal atrophy in BD. We compared brain structure using optimized voxel-based morphometry of 1.5T MRI scans in 33 BD subjects with impaired glucose metabolism (19 with insulin resistance/glucose intolerance (IR/GI), 14 with T2DM), 15 euglycemic BD participants and 11 euglycemic, nonpsychiatric controls. The group of BD patients with IR, GI or T2DM had significantly smaller hippocampal volumes than the euglycemic BD participants (corrected $p = 0.02$) or euglycemic, nonpsychiatric controls (corrected $p = 0.004$). Already the BD subjects with IR/GI had smaller hippocampal volumes than euglycemic BD participants ($t(32) = -3.15$, $p = 0.004$). Age was significantly more negatively associated with hippocampal volumes in BD subjects with IR/GI/T2DM than in the euglycemic BD participants ($F(2, 44) = 9.96$, $p = 0.0003$). The gray matter reductions in dysglycemic subjects extended to the cerebral cortex, including the insula. In conclusion, this is the first study demonstrating that T2DM or even prediabetes may be risk factors for smaller hippocampal and cortical volumes in BD. Abnormal glucose metabolism may accelerate the age-related decline in hippocampal volumes in BD. These findings raise the possibility that improving diabetes care among BD subjects and intervening already at the level of prediabetes could slow brain aging in BD.
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INTRODUCTION

Brain alterations in bipolar disorders (BD) may be secondary to the accumulation of certain clinical variables (Berk *et al*, 2011; Hajek *et al*, 2005). Identifying the relevant clinical factors might allow us to prevent or alleviate some of the brain changes in BD.

Hippocampal volumes are typically preserved in unaffected subjects at genetic risk for BD (McDonald *et al*, 2006; Velakoulis *et al*, 2006; Hajek *et al*, 2009; Karchemskiy *et al*, 2011) or in patients early in the course of the illness (Velakoulis *et al*, 2006; Hajek *et al*, 2009). In contrast, participants with established BD, especially those with substantial illness burden, often display smaller hippocampal volumes than controls (Beyer *et al*, 2004; Hajek *et al*, 2012c, 2012b). Hippocampal atrophy may not be an inevitable outcome of BD, as it is not found in all studies. Even patients with major illness burden may have comparable (Delaloye *et al*, 2009; van Erp *et al*, 2012; Hajek *et al*, 2012b, 2014) or even larger hippocampal volumes than controls (Hajek *et al*, 2012c). Yet, the specific clinical factors associated with abnormal hippocampal volumes in BD remain mostly unknown.

The hippocampus is particularly sensitive to homeostatic changes, including variations in glucose levels (McCrimmon *et al*, 2012). Consequently, participants with type 2 diabetes mellitus (T2DM) typically display smaller hippocampal volumes than nondiabetic controls (den Heijer *et al*, 2003; Gold *et al*, 2007; Bruehl *et al*, 2009; Wrighten *et al*, 2009; Brundel *et al*, 2010; Hayashi *et al*, 2011). The hippocampal volume alterations may occur already in participants with impaired glucose tolerance (Convit *et al*, 2003) or insulin resistance (IR, Rasgon *et al*, 2011; Willette *et al*, 2013). The brain changes in T2DM or IR may not be isolated to the hippocampus, as suggested by some studies (Gold *et al*, 2007; Bruehl *et al*, 2009; Brundel *et al*, 2010; Rasgon *et al*, 2011; Willette *et al*, 2013), but may extend into cortical regions (Benedict *et al*, 2012; Moran *et al*, 2013; Willette *et al*, 2013; Garcia-Casares *et al*, 2014).

Patients with BD have two to three times increased risk of T2DM (Calkin *et al*, 2013b) or metabolic syndrome (Vancampfort *et al*, 2013), which is typically associated with IR. Therefore, it is possible that hippocampal or even cortical atrophy in BD could in part be related to comorbid T2DM or prediabetes. This would be of clinical relevance,

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as T2DM-related neuronal damage can be prevented (Hemmingsen *et al*, 2011) or successfully treated (Chen *et al*, 2013). However, no studies have directly investigated whether comorbid T2DM or prediabetes could explain some of the brain changes found in BD. Studying patients with prediabetes or T2DM and BD could help identify preventable risk factors for neuroimaging abnormalities in BD and could provide insight into the pathophysiology and possible treatment of these alterations.

To address these issues, we systematically evaluated glucose tolerance, IR and obtained structural brain scans from euglycemic BD participants, BD subjects with prediabetes/T2DM and euglycemic, nonpsychiatric controls. We hypothesized that impaired glucose metabolism in BD would be associated with smaller hippocampal volumes. We also explored whether BD participants with dysglycemia would display accelerated age or illness burden-related brain changes. Last, we tested whether the changes associated with prediabetes/T2DM would extend beyond the hippocampus.

MATERIALS AND METHODS

This was a cross-sectional study of BD subjects recruited from the Maritime Bipolar Registry (Ruzickova *et al*, 2003) and our ongoing clinical study of T2DM in BD (Calkin *et al*, 2013a). Controls were recruited through advertisement. The study was approved by the Ethics Committee of Capital District Health Authority and all included subjects signed the informed consent.

Inclusion/Exclusion Criteria

The subjects with BD were required to (1) have a diagnosis of bipolar I or II disorder made by a psychiatrist and (2) be at least 18 years of age. Patients were excluded if they had (1) the diagnosis of organic mood disorder, (2) a mood disorder not otherwise specified, or (3) more than one lifetime course of electroconvulsive therapy (ECT) or ECT within the last 6 months. Control subjects were excluded if they had (1) a personal history of psychiatric disorders or (2) T2DM established based on fasting glucose levels during preventive medical examinations. Subjects from any group were excluded if they (1) met any MRI exclusion criteria or suffered from; (2) substance abuse in the last 12 months, (3) cerebrovascular disease, (4) neurodegenerative disorders, or (5) macrovascular complications of DM, including stroke, as we were interested in the more subtle T2DM-related neuronal changes.

Diagnostic Assessments

For a detailed description of the diagnostic assessment, please see (Ruzickova *et al*, 2003). Briefly, the diagnostic interviews were performed by pairs of clinicians, according to the Schedule for Affective Disorders and Schizophrenia, Lifetime version (SADS-L) (Endicott and Spitzer 1978). We also used NIMH life charts (NIMH-LCM) and assessed the current level of functioning using the Global Assessment of Functioning (GAF) scale. Diagnostic information was reviewed in a blinded fashion in consensus meetings, which included a minimum of two psychiatrists. Each control subject also underwent the SADS-L interview and was included if found to have no personal or family history of Axis I psychiatric disorders. We also collected detailed information about the personal history of medical conditions and smoking. We used the FINnish Diabetes RIsk SCore (FINDRISC) to ascertain diet and exercise (Saaristo *et al*, 2005). This tool, which emphasizes the importance of lifestyle, was developed for population screening of unrecognized T2DM and high diabetes risk.

Diagnosis of IR and T2DM

All patients who did not have a preexisting diagnosis of T2DM with evidence of treatment had fasting plasma glucose (FPG) and fasting serum insulin (FSI) tests performed and analyzed in a single laboratory with the same assay to eliminate variability. If FPG was elevated (>7 mmol/l), the test was repeated on another day to confirm the diagnosis of T2DM. If the diagnosis of T2DM was equivocal (the repeated test was not >7 mmol/l, or the initial FPG was between 5.7 and 6.9 mmol/l), then a 2-h 75 g oral glucose tolerance test was performed. Glucose intolerance (GI) was defined by a FPG <7.0 mmol/l and a glucose level >7.8 and <11.1 mmol/l measured 2 h after ingestion of 75 g of glucose. The diagnosis of T2DM was made if the 2-h glucose level was >11.1 mmol/l, irrespective of FPG. These are standard diagnostic procedures for T2DM. In patients who did not meet the laboratory criteria for T2DM or GI, IR was estimated using the homeostatic model assessment-insulin resistance (HOMA-IR) equation:

$$\text{HOMA-IR} = \text{FPG}\,(\text{mmol/l}) \times \text{FSI}\,(\mu\text{U/ml}) \,/\, 22.5$$

The HOMA-IR strongly correlates with estimates using the euglycemic clamp method (Katsuki *et al*, 2001; Wallace *et al*, 2004) and therefore is a well-accepted measure of IR. We used a HOMA-IR $\geqslant 2.0$ to define IR (Sinha *et al*, 2009). The participants with IR and GI were combined into a single group (BD + IR/GI group) for the analyses. We performed neuroimaging in participants with impaired glucose metabolism (BD + IR/GI and BD + T2DM), who were matched to euglycemic BD subjects and nonpsychiatric euglycemic controls by age and sex.

MRI Methods

MRI acquisition parameters. All MRI acquisitions were performed with a 1.5 Tesla General Electric Signa scanner and a standard single-channel head coil. After a localizer scan, a T1-weighted SPGR (Spoiled Gradient Recalled) scan was acquired with the following parameters: flip angle = 40 degrees, TE = 5 ms, TR = 25 ms, FOV = 24 cm × 18 cm, matrix = 256 × 160 pixels, NEX = 1, no inter-slice gap, 124 images with 1.5-mm slice thickness.

VBM data processing. As in our previous study (Hajek *et al*, 2012b) structural data were analyzed with FSL-VBM, an optimized voxel-based morphometry style analysis carried out with FMRIB's Software Library (FSL), FSL Tools. First, structural images were brain-extracted using

the Brain Extraction Tool. Next, tissue-type segmentation was carried out using FMRIB's Automated Segmentation Tool, version 4. Overall gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) volumes in native space were obtained at this step. The resulting gray matter partial volume images were then aligned to Montreal Neurological Institute (MNI), 152 standard space using the affine registration tool FLIRT, followed by nonlinear registration using FNIRT, which uses a b-spline representation of the registration warp field. The resulting images were averaged to create a study-specific template, to which the native gray matter images were then nonlinearly reregistered. Creating a study-specific template further reduces any potential bias for spatial normalization. As a result of nonlinear spatial normalization, the volumes of certain brain regions may grow, whereas others may shrink. In order to preserve the volume of a particular tissue within a voxel, the registered partial volume images were then modulated by dividing by the Jacobian of the warp field to correct for local expansion or contraction. In effect, an analysis of modulated data tests for regional differences in the absolute amount (volume) of gray matter and controls for whole-brain volume. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm.

Statistical Analyses

For comparisons of clinical and demographic variables, we used one-way ANOVA or *t*-test for continuous variables and the χ^2-test for categorical variables.

Voxel-wise paired GM differences for data preprocessed in FSL were determined using FSL, 'randomize', a permutation-based, non-parametric program, which enables modeling and inferences on statistical parametric maps with an unknown null distribution using a general linear model design, thus requiring no assumptions about the underlying distributions. We selected the hippocampus as the *a priori* region of interest, using WFU PickAtlas version 2.5 (http://fmri.wfubmc.edu/software/PickAtlas), as well as performed whole-brain analyses. We applied a threshold of $p<0.05$ corrected for multiple comparison using the threshold-free cluster enhancement. This technique does not use a specific threshold but takes into account both the spatial extent and height of any between-group differences.

All results are presented using MNI coordinates. To investigate whether impaired glucose metabolism in BD was associated with brain alterations, we used three contrasts. Specifically, we compared (1) euglycemic BD *vs* BD subjects with impaired glucose metabolism (IR, GI, or T2DM), (2) euglycemic controls *vs* BD subjects with impaired glucose metabolism (IR, GI, or T2DM), and (3) euglycemic controls *vs* euglycemic BD participants. We also concurrently covaried for variables known to be associated with hippocampal volumes, including age, sex, and BMI.

We extracted the mean values from the clusters of significant differences between the groups. These were used to calculate effect size (Cohen *d*) and for exploratory and confounder analyses.

To explore whether changes were present already at the level of prediabetes, we compared the BD + IR/GI participants with the combined group of euglycemic participants (euglycemic BD and euglycemic controls subjects) using an independent samples *t*-test.

To investigate the association between illness burden and hippocampal volumes, we used stepwise multiple linear regression with hippocampal volume as the dependent variable and age, duration of illness, number of episodes, and number of hospitalizations as the predictors. For the variables significantly associated with hippocampal volumes, we compared the regression slopes between the IR/GI/T2DM *vs* euglycemic participants using the F ratio.

To control for lifestyle variables (diet, exercise, and smoking), presence of conditions frequently co-occurring with T2DM (hypertension, dyslipidemia, and obesity) and exposure to medications known to affect brain structure (antipsychotics, Li), we compared the groups after separately excluding subjects who performed <30 min of exercise/day, those not eating fruits/vegetables daily, smokers, those with dyslipidemia or hypertension, BMI <25, as well as participants with current antipsychotics or Li treatment. In addition, we performed one-way analysis of covariance comparing euglycemic controls, euglycemic BD subjects, and BD subjects with abnormal glucose metabolism (IR/GI or T2DM), while covarying for diet, exercise, and smoking. We also compared hippocampal volumes in subjects with versus without dyslipidemia or hypertension using independent samples *t*-test.

For these hypotheses generating and exploratory/confounder analyses, we used an uncorrected threshold of $p<0.05$.

RESULTS

Description of the Participants

We analyzed data from 59 subjects: 33 participants with BD and impaired glucose metabolism (4 with GI, 15 with IR and 14 with T2DM), 15 euglycemic BD patients, and 11 age and sex matched euglycemic, psychiatrically healthy controls. The participants with impaired glucose metabolism (IR/GI/T2DM) had higher BMI, a greater proportion of participants with dyslipidemia, hypertension, and a lower GAF score than the other groups (see Table 1). The average age at T2DM diagnosis was 46.0 years (standard deviation (SD) = 9.1). The BD + IR/GI subjects had an average HOMA-IR score of 4.06 (SD = 3.3) and the BD + T2DM subjects had an average fasting glucose of 8.3 mmol/l (SD = 3.5). The groups were comparable in other relevant variables listed in Table 1.

Hippocampal Volumes

BD patients with impaired glucose metabolism (IR, GI, or T2DM) displayed significantly smaller gray matter volumes in the right hippocampus relative to euglycemic BD participants (corrected $p<0.05$, 36 voxels, maximum difference at $x=16$, $y=-30$, $z=-6$, $t=4.30$, corrected p max = 0.02, Cohen $d=-1.3$, 95% confidence interval (CI) = -2.0; -0.7) and in the bilateral hippocampus relative to euglycemic, nonpsychiatric controls (right hippocampus corrected $p<0.05$, 350 voxels, maximum difference at $x=20$, $y=-32$, $z=-10$, $t=4.82$, corrected p max = 0.004, Cohen $d=-1.4$, 95% CI = -2.2; -0.7; left

Table 1 Description of the Sample

	Euglycemic controls $N=11$	Euglycemic BD $N=15$	BD with IR/GI/T2DM $N=33$	*P*
Sex females, *N* (%)	7 (63.6)	12 (80.0)	17 (51.5)	NS
Age, mean (SD) years	43.1 (10.4)	48.3 (8.7)	51.6 (12.3)	NS
Body mass index, mean (SD) kg/m^2	28.4 (4.8)	27.2 (4.4)	31.8 (5.2)	0.01
History of dyslipidemia, *N* (%)	0 (0.0)	0 (0.0)	15 (45.5)	<0.001
History of hypertension, *N* (%)[a]	0 (0.0)	7 (46.7)	20 (64.5)	0.04
Total brain volume, mean (SD) mm^3	1413254.6 (139978.4)	1422130.9 (117529.2)	1395920.7 (188877.5)	NS
Total brain GM volume, mean (SD) mm^3	603848.9 (65897.3)	595144.6 (69383.4)	546809.4 (91934.1)	NS
Smokers, *N* (%)[b]	1 (11.1)	2 (14.3)	9 (28.1)	NS
Performs <30 minutes of exercise/day, *N* (%)[b]	2 (22.2)	2 (15.4)	9 (27.3)	NS
Diet does not contain vegetables and fruits every day, *N* (%)[b]	1 (11.1)	4 (30.8)	16 (48.5)	NS
Diagnosis BD type I, *N* (%)	N/A	9 (60.0)	23 (69.7)	NS
Li at the time of scanning, *N* (%)	N/A	10 (66.7)	18 (54.5)	0.04
Lifetime history of exposure to Li, *N* (%)	N/A	11 (73.3)	27 (81.8)	NS
Antipsychotics at the time of scanning, *N* (%)	N/A	2 (13.3)	12 (36.4)	NS
Lifetime history of exposure to antipsychotics, *N* (%)	N/A	7 (46.7)	22 (66.7)	NS
Personal history of psychotic symptoms, *N* (%)	N/A	7 (46.7)	17 (51.5)	NS
Duration of illness, mean (SD) years	N/A	27.4 (9.5)	27.9 (11.9)	NS
Number of episodes, mean (SD)	N/A	10.0 (9.3)	7.8 (9.7)	NS
Number of hospitalizations, mean (SD)	N/A	1.4 (1.3)	1.6 (1.7)	NS
Childhood Experience of Care and Abuse Score, mean (SD)[c]	N/A	4.2 (4.4)	2.5 (2.8)	NS
Global assessment of functioning, mean (SD)	N/A	75.5 (10.6)	67.6 (10.7)	0.02

Abbreviations: BD, bipolar disorders; IR/GI, insulin resistance, glucose intolerance; Li, lithium; NS non-significant; SD, standard deviation; T2DM, type 2 diabetes mellitus.
[a]Data missing in nine participants.
[b]Data missing in four participants.
[c]Data missing in eighteen participants.

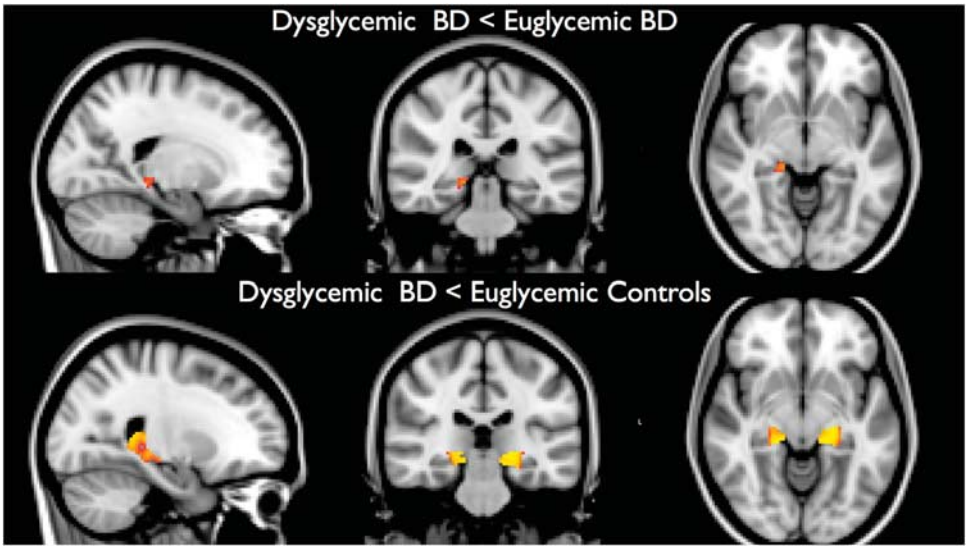

Figure 1 Differences between the groups in hippocampal volumes. A red/yellow cluster denotes a significantly smaller hippocampal volume in dysglycemic relative to euglycemic participants (corrected $p<0.05$).

hippocampus $p<0.05$, 443 voxels, maximum difference at $x=-18$, $y=-30$, $z=-10$, $t=4.15$, corrected p max = 0.004, Cohen $d=-1.4$, 95% CI $=-2.1; -0.6$; see Figure 1). The between-group differences remained significant when we concurrently covaried for age, sex, and BMI ($F(2, 53)=4.57$, $p=0.02$).

There were no significant voxel-wise differences (increases or decreases) within the left or right hippocampal

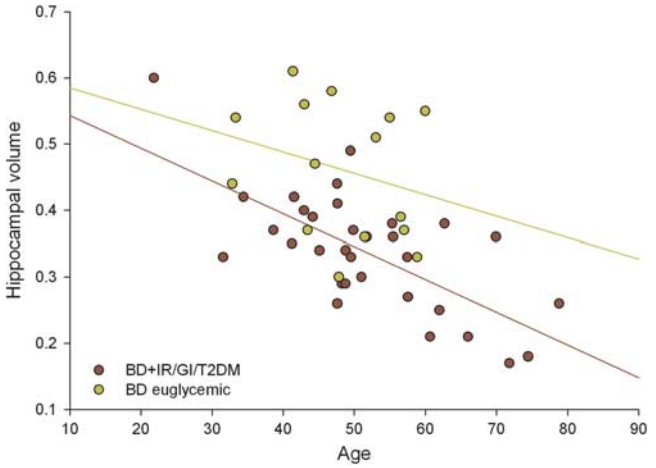

Figure 2 Association between hippocampal volume and age in dysglycemic and euglycemic BD participants.

mask between the euglycemic BD *vs* euglycemic control participants, even at a trend level (corrected $p = 0.10$).

Exploratory and Confounder Analyses

Within the cluster of differences between the dysglycemic and euglycemic subjects, the IR/GI subjects displayed comparable hippocampal GM volumes to T2DM subjects ($t(31) = 1.71$, NS) and smaller volumes than euglycemic BD participants ($t(32) = -3.15$, $p = 0.004$) or euglycemic controls ($t(28) = -3.68$, $p = 0.001$).

In a model containing age, duration of illness, number of episodes, number of hospitalization, only age was significantly associated with hippocampal volumes ($\beta = -0.55$, $t(46) = 10.44$, $p < 0.0001$) and explained 30% of the variance in the hippocampal volumes ($R^2 = 0.30$, $F(1, 46) = 19.50$, $p = 0.00006$). Age was significantly negatively associated with hippocampal volumes in dysglycemic BD subjects ($r(31) = -0.68$, $p = 0.00001$) but not in euglycemic BD participants ($r(13) = -0.27$, NS), yielding a significant difference between these two regression slopes ($F(2, 44) = 9.96$, $p = 0.0003$, see Figure 2).

The differences between the euglycemic BD and dysglycemic BD subjects remained significant when we excluded subjects currently treated with Li ($t(18) = 2.49$, $p = 0.02$) or antipsychotics ($t(32) = 4.73$, $p < 0.0001$) or when we limited the analyses to subjects with lifetime history of exposure to Li ($t(36) = 3.40$, $p = 0.002$) or antipsychotics ($t(27) = 2.19$, $p = 0.04$).

BD patients with impaired glucose metabolism (IR, GI, or T2DM) had significantly smaller hippocampal volumes than the euglycemic groups even when covarying for diet, exercise, and smoking ($F(2, 48) = 5.72$, $p = 0.006$) or in subgroup analyses limited to subjects performing at least 30 min of exercise/day ($F(2, 39) = 7.88$, $p = 0.001$), those eating fruits/vegetables on a daily basis ($F(2, 31) = 10.9$, $p = 0.0003$), nonsmokers ($F(2, 40) = 6.09$, $p = 0.005$), those without dyslipidemia ($F(2, 41) = 3.55$, $p = 0.04$) or hypertension ($F(2, 20) = 6.26$, $p = 0.008$), or those with a BMI > 25 ($F(2, 42) = 7.66$, $p = 0.002$). Dysglycemic BD subjects with dyslipidemia had smaller hippocampal volumes than dysglycemic BD subjects without dyslipidemia ($t(31) = -3.35$, $p = 0.002$). There was no such association for hypertension ($t(29) = 1.47$, $p =$ NS).

Whole-Brain Analyses

The whole-brain analyses revealed significantly smaller GM volumes in BD patients with impaired glucose metabolism (IR, GI, or T2DM) relative to euglycemic controls or euglycemic BD subjects, with maxima in the frontal lobe, parietal lobe, thalamus, and cerebellum (Table 2), but extending to the insula and basal ganglia, see Figure 3. There were no significant increases in GM volumes in dysglycemic subjects relative to the euglycemic BD or euglycemic control subjects.

DISCUSSION

This is the first study demonstrating that T2DM or even prediabetes may be risk factors for brain structural alterations in BD. Specifically, BD patients with IR/GI or T2DM had smaller hippocampal volumes than euglycemic BD participants, who had comparable hippocampal volumes to euglycemic controls. Already the prediabetic BD subjects had significantly smaller hippocampal volumes than the euglycemic participants. In addition, patients with IR/GI or T2DM displayed accelerated age-related decline in hippocampal volumes. Last, the GM changes associated with impaired glucose metabolism extended to wide areas of cerebral cortex. These findings are congruent with our *a priori* hypotheses and with previous reports of hippocampal (den Heijer *et al*, 2003; Gold *et al*, 2007; Bruehl *et al*, 2009; Wrighten *et al*, 2009) or cortical atrophy (Benedict *et al*, 2012; Moran *et al*, 2013; Willette *et al*, 2013; Garcia-Casares *et al*, 2014) in patients with T2DM or IR. Furthermore, the effect sizes observed in this study (1.3–1.4) were comparable to large effect sizes (0.8–1.4) reported in other studies comparing hippocampal volumes between participants with versus without T2DM (Gold *et al*, 2007; Bruehl *et al*, 2009; Brundel *et al*, 2010).

When interpreting the results, we need to consider potential confounding by clinical, treatment, and lifestyle factors, which may be associated with dysglycemia and may have adverse effects on the brain. Importantly, the differences between the euglycemic and dysglycemic participants persisted when we controlled for exposure to Li, antipsychotics, BMI, personal history of dyslipidaemia, cardiovascular disease, hypertension, diet, exercise, and smoking. In addition, the groups were comparable in early adversity and the changes were not isolated to the hippocampus as could be expected for stress-related alterations. Therefore, the most parsimonious explanation continues to be that the observed alterations were related to between-group differences in glucose metabolism.

Cross-sectional and some prospective studies have suggested that structural neuroimaging changes in BD may accumulate over time (Lim *et al*, 2013) or throughout the course of illness (Berk *et al*, 2011; Hajek *et al*, 2012a). In keeping with this, BD participants with IR/GI/T2DM displayed accelerated age-related decline in hippocampal volumes relative to euglycemic subjects. This finding could

help explain why smaller hippocampal volumes are typically not found in young unaffected relatives of BD participants (McDonald *et al*, 2006; Velakoulis *et al*, 2006; Hajek *et al*, 2009; Karchemskiy *et al*, 2011) or in young BD patients (Velakoulis *et al*, 2006; Hajek *et al*, 2009), who have a low risk of T2DM, but often occur in subjects with longer duration of illness (Beyer *et al*, 2004; Hajek *et al*, 2012c, 2012b), who are at an age range where T2DM becomes more prevalent.

The neuroanatomical changes associated with impaired glucose tolerance were not isolated to the hippocampus, but extended into the cerebral cortex, including the frontal, parietal and temporal lobes, the insula, and cerebellum. Volumetric changes in these regions have previously been reported in BD (Selvaraj *et al*, 2012) and have been associated with specific BD-related cognitive functions (Delvecchio *et al*, 2012). The widespread gray matter alterations may explain why patients with BD complicated by T2DM or metabolic syndrome display adverse clinical or treatment outcomes (Ruzickova *et al*, 2003; McIntyre *et al*, 2010). Interestingly, one of the most affected regions in the dysglycemic BD subjects was the insular cortex, which has a critical role in interoception and homeostatic monitoring and regulation (Craig 2002).

We can only speculate about the mechanisms underlying these global volumetric changes. The cognitive and brain alterations in T2DM may be associated with hyperglycemia (glucose toxicity) or IR (McCrimmon *et al*, 2012). We found alterations already at the level of IR, when subjects displayed a combination of euglycemia and hyperinsulinemia. This may suggest that hyperglycemia-related effects, such as elevation in advanced glycation endproducts or microangiopathy (Orasanu and Plutzky 2009), may not be necessary for these brain changes to occur. Furthermore, the fact that the changes extended beyond the hippocampus to cortical regions makes them less likely to be related to stress associated with T2DM. Perhaps these alterations are predominantly associated with impaired insulin signaling, with resulting withdrawal of trophic factors, inhibition of insulin-responsive gene expression and impaired mitochondrial energy metabolism, which increases oxidative stress (Brietzke *et al*, 2011; Hajek *et al*, 2013).

The results are clinically concerning as T2DM remains underdiagnosed and IR is not even screened for in BD patients. The association between dysglycemia and brain structure emphasizes the need to improve diabetes care in BD and to implement collaborative chronic care models, which would better integrate psychiatric and medical management (Unutzer *et al*, 2006; Miller *et al*, 2013). Identification of IR/GI/T2DM as potential risk factors for structural brain changes in BD may be the first step toward their management, especially as T2DM-related neuronal

Table 2 Regional Differences Between the Groups from the Whole-Brain Voxel-Based Morphometry Analyses

Region, Brodmann area (BA)	*x,y,z* (MNI coordinates)	*t*	corrected *p*	*N* voxels
Dysglycemic BD > euglycemic BD				
None				
Dysglycemic BD < euglycemic BD				
L postcentral gyrus, BA 2	−58, −16,30	4.85	0.007	3319
R precentral gyrus, BA 6	46, −8,26	5.59	0.004	801
R inferior parietal lobule, BA 40	42, −28,48	4.81	0.024	105
Dysglycemic BD > euglycemic controls				
None				
Dysglycemic BD < euglycemic controls				
L thalamus	−22, −28,4	6.77	0.0004	27 773
R cerebellum, pyramis	30, −90, −32	4.44	0.032	183
R medial frontal gyrus, BA 6	22, −10,50	4.13	0.041	36
R middle temporal gyrus, BA 21	44, −54,4	4.75	0.044	25
R cerebellum, tonsil	12, −62, −36	4.34	0.045	22

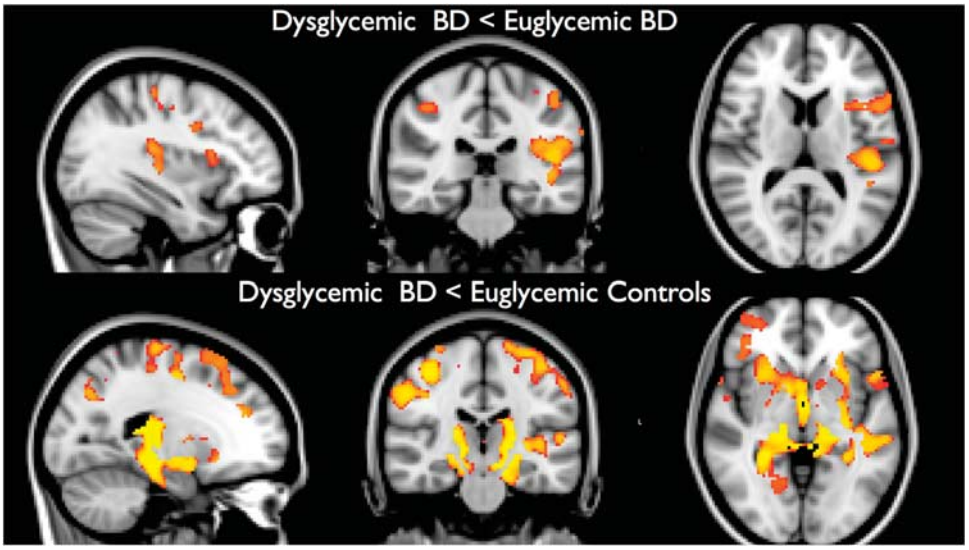

Figure 3 Regional differences between the groups from the whole-brain voxel-based morphometry analyses. A red/yellow cluster denotes a significantly smaller gray matter volume in dysglycemic relative to euglycemic participants (corrected $p < 0.05$).

tissue damage can be alleviated (Hemmingsen *et al*, 2011; Chen *et al*, 2013). It is important to test whether improving diabetes care in BD would improve these neuroimaging alterations and slow down the accelerated brain aging in BD. These findings also justify intervening already at the stage of IR. Last, it is important to test whether insulin sensitizers or antioxidants can alleviate brain changes in BD. This would allow us to expand the limited psychiatric pharmacopoeia, as well as focus the treatment of BD on biological outcome measures rather than just behavioral symptoms.

Our results also provide novel leads with potential clinical implications. Previous studies have documented that body mass index negatively correlates with brain volumes in BD (Bond *et al*, 2011, 2014). In this study, the between-group differences in hippocampal volumes remained significant when we covaried for BMI. In addition, overweight participants with impaired glucose metabolism displayed smaller hippocampal volumes than overweight participants with euglycemia. Therefore, our results extend the findings of Bond *et al* (2011, 2014), to show that impaired glucose metabolism in BD may contribute to the brain changes observed in overweight subjects. We also found the smallest hippocampal volumes in dysglycemic subjects with dyslipidemia. Future studies should investigate the combined effects of psychiatric disorders and dyslipidemia on the brain. This is particularly important considering the increased risk of metabolic syndrome not only in BD but also in other psychiatric disorders.

This study has several limitations. A prospective study would have better allowed us to establish the causality of the association between T2DM and brain structure. We did not include T2DM or IR/GI patients without BD or patients with other psychiatric disorders. Therefore, the relative contribution of each condition to the observed changes or the specificity of these effects to BD remains unclear. The euglycemic control group was relatively small. On the other hand, with 59 subjects this study was sufficiently powered to detect effect sizes similar to those reported previously (Gold *et al*, 2007; Bruehl *et al*, 2009; Brundel *et al*, 2010). A growing body of evidence suggests that brain structure may change as a consequence of treatment with Li (Hajek *et al*, 2012a) or antipsychotics (Fusar-Poli *et al*, 2013). Recruiting medication naive subjects would not have been feasible, as T2DM typically develops many years after the onset of BD (Table 1). The results remained essentially unchanged when we controlled for current or lifetime treatment with Li or antipsychotics. Although we did not have detailed information about long-term glycemic control (HbA1c), the differences found in nondiabetic subjects with IR could not have been related to poor glucose control.

The advantages of this study include the systematic biochemical evaluation, which allowed us to address the underdiagnosis of T2DM in BD and to investigate a group of patients with IR/GI. Insulin resistance typically remains undetected and consequently is also understudied. Collecting detailed clinical information allowed us to control for potential confounders. The fact that the results remained consistent in subgroup analyses or when we controlled for relevant clinical, treatment, or lifestyle variables supports the robustness and validity of these findings.

In conclusion, both T2DM and prediabetes may be risk factors for hippocampal and cortical brain alterations in BD. This is clinically concerning, as BD patients have an increased risk of diabetes and yet often receive suboptimal diabetes care. Even more concerning was the fact that neuroimaging changes were present already in subjects with prediabetes, which is not even monitored for in BD. In addition, abnormal glucose metabolism may accelerate the age-related decline in hippocampal volumes in BD. These findings raise the possibility that improving diabetes care among BD subjects and intervening already at the level of prediabetes could slow brain aging in BD.

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