

Subgenual cingulate volumes in offspring of bipolar parents and in sporadic bipolar patients

Tomas Hajek · Tomas Novak · Miloslav Kopecek ·
Eva Gunde · Martin Alda · Cyril Höschl

Received: 5 February 2009 / Accepted: 25 September 2009 / Published online: 8 October 2009
© Springer-Verlag 2009

Abstract Decreased volumes of subgenual cingulate (SGC) have been reported primarily among familial bipolar patients, which is one of the hallmarks of an endophenotype. In order to investigate specificity of SGC volume abnormalities to familial mood disorders and to test whether SGC volumes represent an endophenotype for BD, we measured SGC volumes in young affected and unaffected relatives of bipolar patients (high-risk design) and in sporadic bipolar patients. We included 20 unaffected, 15 affected offspring of bipolar I or bipolar II parents, 18 controls, and 19 sporadic bipolar patients between 15 and 30 years of age. SGC volumes were measured on 1.5 T 3D anatomical MRI images using standard methods. We also combined the effect sizes from all published studies of sporadic patients with mood disorders ($N = 61$) and controls ($N = 84$) using random-effect models. We found comparable SGC volumes among unaffected, affected offspring of BD parents and controls ($F = 0.7$, $df = 2$; 50 , $P = 0.47$). Likewise no SGC abnormalities were found between sporadic bipolar and control subjects ($F = 2.31$, $df = 1$; 34 , $P = 0.14$). When combining all available data from sporadic patients, there were no differences in left (SDM 0.19, 95% CI -0.13 to 0.51) or right (SDM -0.11 , 95% CI -0.47 to 0.26) SGC volumes between

sporadic bipolar patients and controls. The limitations of the study are cross-sectional design and inclusion of both bipolar I and bipolar II probands. In conclusion, SGC volume abnormalities were absent in unaffected, affected relatives of bipolar patients as well as sporadic bipolar patients and thus did not meet criteria for endophenotype.

Keywords Bipolar disorders · MRI · Subgenual cingulate · High-risk

Introduction

Converging lines of evidence suggest, that subgenual cingulate (SGC), the first full gyrus beneath the genu of corpus callosum, is implicated in regulation of emotions [25] and plays an important role in the pathophysiology of mood disorders. Patients with unipolar (UD) or bipolar (BD) disorders have reduced glial counts [24] and lower volumes of SGC [15]. Functional neuroimaging investigations showed increased SGC glucose uptake or perfusion during induction of sadness [21] or depression [22] and normalization of this pattern during successful treatment of depression with various antidepressant modalities [9, 20]. Based on these studies deep brain stimulation of white matter tracts adjacent to SGC has successfully been used to alleviate treatment refractory depression [22].

Volumetric studies of SGC show marked heterogeneity which likely stems from clinical, demographic, as well as MRI-related issues [15, 18]. It is for example still not clear whether structural changes within SGC represent biological risk factors (endophenotypes in a narrower sense) or whether they are secondary to burden of illness, comorbid conditions or treatment [11]. This distinction is important from the research as well as clinical perspectives, as

T. Hajek (✉) · M. Alda
Department of Psychiatry, Dalhousie University, QEII HSC,
A.J.Lane Bldg., Room 3093, 5909 Veteran's Memorial Lane,
Halifax, NS B3H 2E2, Canada
e-mail: tomas.hajek@dal.ca

T. Hajek · E. Gunde
Department of Anatomy and Neurobiology,
Dalhousie University, Halifax, NS, Canada

T. Hajek · T. Novak · M. Kopecek · M. Alda · C. Höschl
Prague Psychiatric Centre, 3rd Faculty of Medicine,
Charles University, Prague, Czech Republic

endophenotypic changes are well suited for genetic investigations and may be used for early objective diagnosis, whereas changes secondary to the burden of illness may be targets for preventive measures. Co-segregation of a biological abnormality with illness in families is one characteristic of an endophenotype. Recent meta-analysis of SGC volumes in mood disorders showed that decreased SGC is predominantly found in subjects with family history of mood disorders [15]. None of the studies in sporadic subjects found abnormal SGC volumes. However, the studies of sporadic patients were typically underpowered with small numbers of participants (range 6–15) [2, 17, 27].

In order for a particular biological change to be considered an endophenotype, presence among unaffected subjects at genetic risk for developing an illness needs to be demonstrated [10]. Such high-risk (HR) design (study of offspring of bipolar parents) is crucial for other reasons as well. There is an emerging evidence from prospective follow-up studies of bipolar offspring, that bipolar disorders develop in distinct clinical stages [5]. It appears that non-specific psychopathology such as anxiety or sleep disorders among offspring of bipolar patients may in fact represent early stages of bipolar disorders. Little is known about the biological underpinnings of such staging. In light of these facts, there is a great need for studies of early bipolar disorders. The best way to bypass the diagnostic controversies and to study early development of BD is performing a HR study of children of bipolar parents.

We previously published the first study of SGC volumes among affected as well as unaffected relatives of bipolar patients [12]. SGC volumes in this study were comparable among the groups. In order to further elucidate the role of SGC in development of BD, we now present the second independent study of SGC volumes among offspring of bipolar parents. If SGC volumes are an endophenotype for BD, then affected offspring should show significantly lower SGC volumes relative to controls with unaffected subjects having intermediate volumes. In order to assess specificity of SGC changes to familial patients, we also included a group of bipolar patients without family history of mood disorders. This is the second HR study of SGC volumes in mood disorders and the largest study of SGC volumes in sporadic bipolar patients.

Methods

Subjects

Probands

The HR offspring were identified through adult probands with bipolar I or II disorders who participated in Czech

Bipolar Disorder Case Registry (CZ-BDCR). CZ-BDCR is a database of patients with bipolar disorder confirmed by Schedule for affective disorders and schizophrenia—lifetime version (SADS-L) interview [8] when entering. The participants are then prospectively followed up. Thirty-two families in which one parent was affected with bipolar disorder (27 BD I, 5 BD II), participated in this study. In nine cases the parent was not available for direct interview (death, health complications). Diagnosis of the parent in these instances was established based on available hospital charts and information from treating psychiatrist and family members.

Offspring of bipolar parents

The offspring of bipolar parents were interviewed using SADS-L interview conducted by experienced research psychiatrists (T.N, M.K.) and where available, hospital records were also reviewed. The offspring of bipolar parents were divided into two subgroups: (1) Unaffected offspring with no Axis I diagnosis, $N = 20$. These subjects were considered to be at an increased risk for development of BD, because they had one parent affected with BD. (2) Offspring of bipolar parents affected with Axis I diagnosis of mood disorders, i.e. personal history of at least one episode of depression, hypomania or mania meeting full DSM IV criteria, $N = 15$ (5 BD I, 4 BD II and 6 UD).

Sporadic patients

Nineteen patients (12 females) with sporadic bipolar disorder were identified through hospital database and outpatient clinics at the Prague Psychiatric Centre. Each thus identified subject underwent SADS-L interview conducted by experienced research psychiatrists (T.N, M.K.) Negative psychiatric family history was evaluated by acquiring family history from the patient and if possible also from one of the parents. We focused on family history of mood disorders in first degree relatives.

Offspring of healthy parents (controls)

Control subjects consisted of 18 healthy offspring of healthy parents recruited through advertisement from similar sociodemographic areas as the patients. Each subject underwent SADS-L interview conducted by experienced research psychiatrists (T.N, M.K.) and was deemed to have no lifetime history of Axis I psychiatric disorders. The control subjects were selected to closely match HR subjects by age and sex. Negative psychiatric family history was evaluated by acquiring family history from the control subject and if possible also from one of the parents.

The inclusion criterion for all groups was age range 15–30 years. Subjects were excluded if they met MRI exclusion criteria (pacemaker, metal implants), had any serious medical illness (e.g. Cushing's disease, conditions treated with corticosteroids), neurological disorder (e.g., epilepsy, head trauma with loss of consciousness, demyelinating disorders) or fulfilled criteria for substance abuse or dependence during last 6 months except nicotine dependence. Additional exclusion criteria for the control group were personal or family history of psychiatric disorders. Subjects were allowed to continue with their psychotropic medications at the time of scanning. All subjects were deemed euthymic during MRI assessment by psychiatrist according to current symptoms description in SADS-L interview, which was conducted ± 1 week from MRI.

After complete description of the study to the subjects, written informed consent was obtained, prior to inclusion in the study. The study was reviewed and approved by The Prague Psychiatric Centre Institutional Review Board and has been performed in accordance with 1964 Declaration of Helsinki.

MRI methods

MRI acquisition parameters

All MR acquisitions were performed with a 1.5 T General Electric Signa scanner and a standard single-channel head coil. After a localizer scan, a T1-weighted SPGR (spoiled gradient) scan was prescribed with the following parameters: flip angle = 40°, TE = 5 ms, TR = 25 ms, field of view = 24 cm \times 18 cm, matrix = 256 \times 160 pixels, NEX = 1, no inter-slice gap, 124 images 1.5 mm thick.

MRI volumetry

Anatomical measurements were conducted using the AFNI software for Linux [3], in a single batch, according to a well-established procedure [4]. Prior to volumetric measurements, all scans were reoriented perpendicular to bicommissural line. Subsequently the gray matter of the first full gyrus beneath the corpus callosum was manually traced in all coronal slices between the most anterior point of the corpus callosum and the slice where the internal capsule no longer divided the striatum. In addition we used the sagittal plane to check for accuracy of the superior inferior landmarks and the axial plane to better delineate the left from the right SGC. Segmentation was performed by one investigator (E.G) blinded to the diagnosis and group assignment of subjects. The intra-class correlation coefficients established by tracing ten scans by two independent raters (E.G, T.H) were $r = 0.95$ for both the right and left SGC (inter-rater reliability). Intra-class correlation

coefficient for ten randomly selected SGCs of the study subjects measured twice by the same rater (E.G) was $r = 0.98$ and $r = 0.97$ for the left and right SGC, respectively (intra-rater reliability).

Calculation of intracranial volumes was performed automatically using 3dAnhist command in AFNI software [3].

Statistical analyses

Following statistical analyses were done using the BMDP (Biomedical Package, Statistical Solutions, Saugus, MA) statistical software. For comparison of offspring of bipolar parents and control subjects, we performed repeated measures analysis of variance (ANOVA) with SGC volumes as the dependent variable, side as the repeated measure and status (affected offspring, unaffected offspring, control subjects) as the grouping variable. We also repeated these analyses with intracranial volumes and age as covariates. Since the sporadic patients differed from healthy controls in age, we performed repeated measures analysis of covariance (ANCOVA) with SGC volumes as the dependent variable, side as the repeated measure, status (sporadic bipolar patients, control subjects) as the grouping variable and age as covariate for comparison of these two groups. We also repeated these analyses with intracranial volumes as covariates. To compare continuous variables (intracranial volumes, age) between three groups (affected offspring, unaffected offspring, control subjects) we used one-way analysis of variance. To compare continuous variables (intracranial volumes, age, duration of illness, duration of treatment, number of episodes) between two groups (sporadic bipolar patients vs. healthy controls or affected offspring of bipolar parents vs. sporadic bipolar patients) we used independent samples *t* tests. Categorical demographic variables (sex, handedness, personal, family history of psychosis, personal history of exposure to medications) were compared using Pearson χ^2 test. To test for association between age, duration of illness, duration of treatment and SGC volumes we used Pearson's correlation coefficient (module 8D in BMDP statistical software). We report nominal, two tailed *P* values.

We performed two types of power analyses. (1) In order to make our study directly comparable to previous studies of familial patients, we calculated number of subjects needed for comparison of two groups in order to achieve 80% statistical power to detect Cohen's *d* (Cohen's $d = M1 - M2/s$ pooled) similar to that found in previous positive studies of affected familial and control subjects. (2) In order to take into account the HR design of this study, we also calculated the minimum effect size detectable as statistically significant for comparisons between three groups of 53 subjects—unaffected offspring, affected

offspring, controls. This effect size was calculated as root mean square standardized effect size (RMSSE), which is the square root of the sum of squared standardized effects divided by the number of degrees of freedom for the effect.

Since none of the previous studies of sporadic subjects showed statistically significant differences between patients and controls at $P = 0.05$, meaning that the 95% confidence intervals for effect size contained 0, calculating a priori power would thus not be meaningful for these comparisons.

In order to maximize power, we also performed a meta-analysis of SGC volumes in available studies of sporadic bipolar patients relative to control subjects using Comprehensive Meta Analysis, Version 2. As a measure of effect size, we also used Cohen's d . Because we cannot expect constant population effect size across studies (fixed effects), we decided to use the random-effects model, with "study" as the random effect. This assumes that the population of studies has variable true effects that are normally distributed. We adopted a significance level of $P = 0.05$, two-tailed for these analyses.

Results

Offspring of bipolar parents versus healthy controls

Demographics

We recruited 20 unaffected offspring, 15 affected offspring and 18 controls and the details are given in Table 1. The groups were matched by age, sex, handedness. The affected and unaffected HR offspring did not differ in sex of the affected parent, parental diagnosis or lifetime history of psychosis among parents.

Volumetric results

There were no differences among the groups in intracranial volumes. Likewise the unaffected, affected offspring of bipolar parents and controls showed comparable SGC volumes ($F = 0.7$, $df = 2$; 50, $P = 0.47$), with no main effect of side ($F = 1.48$, $df = 1$; 50, $P = 0.23$), and no side by group interaction ($F = 0.14$, $df = 2$; 50, $P = 0.87$). The largest effect size of Cohen's $d = 0.39$ was found for a smaller LSGC in affected offspring of bipolar parents relative to controls—for details see Fig. 1. These results remained comparable when we covaried for age or ICV.

We would need 16 subjects in 2 groups to detect Cohen's d of 0.93, the lowest ES reported for SGC volume decrease in previous positive studies, as statistically significant. With 53 subjects we have 80% power to detect RMSSE of 0.55 between 3 groups as statistically significant.

Sporadic bipolar versus control subjects

Demographics

Relative to affected offspring of bipolar parents, the sporadic BD subjects were older, had longer duration of illness, greater current and lifetime exposure to lithium and a trend for differences in several other relevant variables (treatment exposure at the time of scanning, treatment duration, number of manic episodes, number of episodes), for details see Table 1. Because of these differences, which could confound the results, we did not compare these two groups directly but rather performed comparisons between sporadic BD and control subjects. The sporadic BD versus control subjects were comparable in proportion of women, but were significantly older, with no left handed subjects among controls and 4/19 left-handed subjects among sporadic BD patients ($X^2 = 4.2$, $df = 1$, $P = 0.04$).

Volumetric results

There were no differences among the groups in intracranial volumes. Due to differences between groups in age, we covaried for age in subsequent analyses. There was no difference between the sporadic BD and control subjects ($F = 2.31$, $df = 1$; 34, $P = 0.14$), with no effect of side ($F = 0.03$, $df = 1$; 35, $P = 0.87$), or interaction between group and side ($F = 0.06$, $df = 1$; 35, $P = 0.81$). The largest effect size was Cohen's $d = 0.37$ for larger LSGC among sporadic patients relative to controls—for details see Fig. 2. These results remained comparable when we covaried ICV.

None of the previous studies of sporadic bipolar patients showed significant difference in SGC volumes between sporadic bipolar and control subjects. In order to increase statistical power, we combined the effect sizes from this and the previous studies comparing sporadic patients with mood disorders (overall $N = 61$) and controls (overall $N = 84$). There were no differences in SGC volumes between sporadic BPD and control subjects for the left (SDM 0.19, SE 0.16, 95% CI -0.13 to 0.51 , $z = 1.17$, $P = 0.24$) or right SGC (SDM -0.11 , SE 0.18, 95% CI -0.47 to 0.26 , $z = -0.58$, $P = 0.56$), see Fig. 3 for details.

Exploratory analyses

Significantly more sporadic patients had current and lifetime exposure to Li. However, there were no differences between patients with versus without current or lifetime exposure to Li. There were no differences in SGC volumes between unipolar, bipolar I, bipolar II and control subjects. There was no correlation between age, duration of illness,

Table 1 Demographic, clinical and neuroimaging description of included subjects

	Unaffected offspring	Affected offspring	Controls	Affected no FH	<i>P</i> (3 groups: unaffected HR, affected HR, controls)	<i>P</i> (affected HR vs. affected no FH)
<i>N</i>	20	15	18	19	N/A	N/A
Sex, <i>N</i> (%) female	11 (55.0)	11 (73.3)	11 (61.1)	12 (63.2)	NS	NS
Age, years, mean (SD)	20.2 (4.2)	22.1 (4.8)	23.0 (3.5)	26.5 (3.4)	NS	0.004
Age range (years)	15.0–30	15.0–30.0	16.0–29.0	17.0–30.0	N/A	N/A
Diagnosis	N/A	6 MD, 5 BD I, 4 BD II	N/A	16 BD I, 3 BD II	N/A	0.003
Family history (bipolar I parent, bipolar II parent)	16, 4	13, 2	0, 0	0, 0	N/A	N/A
Parent psychosis, <i>N</i> (%)	6 (30)	5 (33.3)	N/A	N/A	N/A	N/A
Treatment at the time of scanning, <i>N</i> (%)	N/A	11 (73.3) (AP = 6, AC = 3, AD = 5, Li = 1)	N/A	18 (94.7) (AP = 11, AC = 8, AD = 2, Li = 8)	N/A	0.08
Treatment duration (month), mean (SD)	N/A	26.7 (36.0)	N/A	50.6 (38.9)	N/A	0.08
Illness duration (month), mean (SD)	N/A	37.9 (41.6)	N/A	75.2 (48.2)	N/A	0.02
<i>N</i> episodes, mean (SD)	N/A	2.8 (3.0)	N/A	4.8 (3.4)	N/A	0.08
<i>N</i> hospitalizations, mean (SD)	N/A	1.8 (1.7)	N/A	2.3 (2.0)	N/A	NS
<i>N</i> manic episodes, mean (SD)	N/A	1.1 (1.9)	N/A	2.1 (1.5)	N/A	0.09
Lifetime history of substance abuse, <i>N</i> (%)	0 (0)	3 (20) (alcohol abuse = 2, cannabis abuse = 1) ^a	0 (0)	1 (5.3) (alcohol abuse) ^a	N/A	NS
Anxiety DO, <i>N</i> (%)	0 (0)	3 (20)	0 (0)	3 (15.8)	N/A	NS
Psychosis ever, <i>N</i> (%)	0 (0)	5 (33.3)	0 (0)	9 (47.4)	N/A	NS
Li ever, <i>N</i> (%)	N/A	3 (20)	N/A	12 (63.2)	N/A	0.01
Li current, <i>N</i> (%)	N/A	1 (6.7)	N/A	8 (42.1)	N/A	0.02
Right handed (%)	93.7	100	100	78.9	NS	0.06
Intracranial volume (mm ³), mean (SD)	1.3520E+6 (182,129.0)	1.2349E+6 (329,649.6)	1.2814E+6 (357,010.7)	1.3347E+6 (126,148.0)	NS	NS
Left SGC volume (mm ³), mean (SD)	305.8 (116.5)	299.2 (100.0)	345.2 (131.5)	396.2 (159.0)	NS	N/A
Right SGC volume (mm ³), mean (SD)	332.2 (87.2)	323.5 (121.5)	353.2 (104.3)	394.7 (107.7)	NS	N/A

^a No subject had a history of substance abuse within the last 12 months. The last episode meeting criteria for substance abuse happened more than 1 year prior to inclusion in the study in all of these subjects

AC Anticonvulsants, AD antidepressants, AP antipsychotics, BD bipolar disorder, Li lithium, MD major depression, mm³ cubic millimeter, N/A not applicable, NS not significant, SD standard deviation, FH family history, HR high risk

duration of treatment, number of episodes, number of hospitalizations and SGC volumes.

Discussion

We found comparable SGC volumes among the affected, unaffected offspring of bipolar parents and controls. With

20 unaffected, 15 affected offspring of bipolar parents and 18 controls, this study was sufficiently powered to detect effect sizes similar to those found in previous positive studies of SGC volumes in bipolar patients [4, 17, 27]. In fact, the largest ES in our present study was 0.39, which is well beneath the previously reported ES (>0.9 and up to 1.3) in previous positive studies of SGC volume in familial bipolar patients [4, 17, 27]. There was not even a trend for

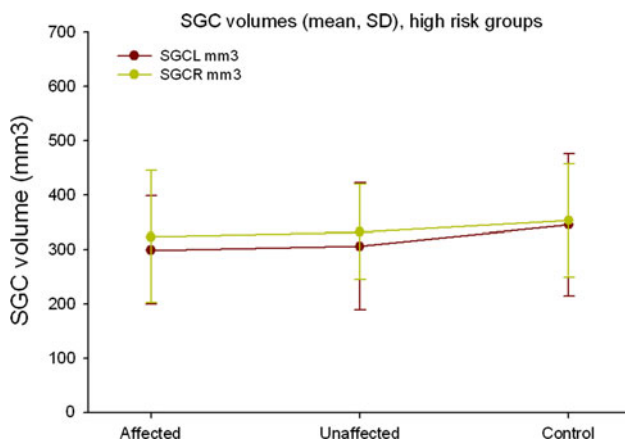


Fig. 1 Left and right subgenual cingulate volumes in offspring of bipolar parents and healthy controls (mean values and standard deviations)

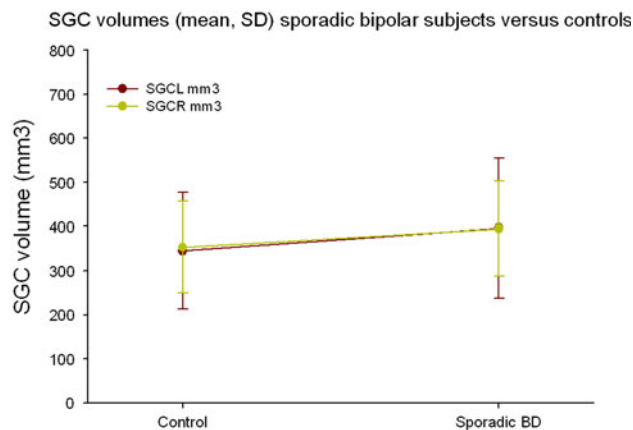
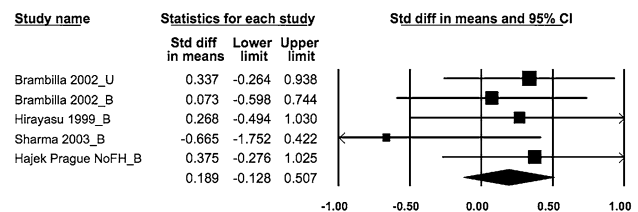


Fig. 2 Left and right subgenual cingulate volumes in sporadic bipolar patients and healthy controls (mean values and standard deviations)

difference and the SGC volume distributions in the three groups markedly overlapped. It is thus unlikely that these results were false negative. When considering the extent of differences, effect size of 0.39 represents 73.3% overlap between two distributions. The biological significance of such a small difference is questionable especially since interpretation of volumetric findings with low effect size in terms of underlying pathophysiology is not clear. In any case, if there are biologically meaningful differences in HR subjects relative to controls, these are only of 1/2 to almost 1/3 extent of changes reported in previous positive studies of familial bipolar subjects.

Our findings are consistent with the single previous HR study of SGC volumes, which also found comparable SGC volumes between unaffected, affected relatives of bipolar patients and healthy controls [12]. They are also congruent with lack of neurocognitive [7, 19] or volumetric changes in other regions of interest [13, 14, 23] among unaffected

LSGC volumes sporadic bipolar patients versus controls



RSGC volumes sporadic bipolar patients versus controls

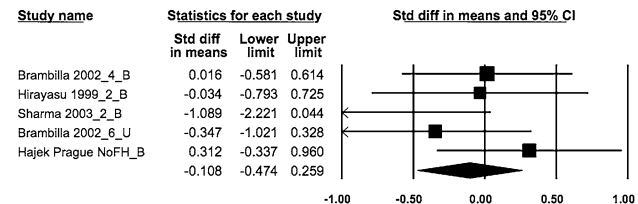


Fig. 3 Forest plot of effect sizes for differences in subgenual cingulate volumes between sporadic patients with mood disorders and healthy controls in previously published and our study. *LSGC* Left subgenual cingulate, *RSGC* right subgenual cingulate, *U* unipolar subjects, *B* bipolar subjects, *FH* family history

relatives of bipolar patients. The lack of changes among affected offspring of bipolar parents is contrary to some [4, 17, 27], but not all [2, 12, 26], previous studies of familial bipolar and unipolar patients. There are notable differences between the present investigation and previous positive reports of decreased SGC among familial patients. These include method of recruitment, age, medication status, diagnostic composition, setting, but also methodological factors regarding SGC volume tracings.

The seminal study of SGC volumes, which also reported the largest SGC volume differences, did not provide detailed information about medication and recruited currently symptomatic (both manic but also depressed) subjects. Pertinent information about burden of illness in these patients, including duration of illness, number of episodes, length of treatment, presence of psychosis were missing, making it difficult to evaluate the extent of differences in clinical burden between this and our study [4]. A second study found smaller SGC volumes in a small sample of six euthymic, mostly medicated familial outpatients with longer duration of illness (average illness length 12 vs. 3.2 years) and older age (average age 38.3 vs. 22.1 years) relative to patients in our study. The authors of that study did not provide information about blinding of raters and about interrater reliability for MRI volumetry of SGC [27]. Manual tracing of region of interest requires subjective input. Blinding and demonstration of replicable method is thus crucial, to prevent experimenter bias. Furthermore, this study was the only investigation to have reported right SGC volume decrease, whereas all other positive studies found left SGC volume abnormalities. The third positive

study reported SGC volume decreases in 14 currently symptomatic, antipsychotic-treated patients hospitalized for psychotic mood disorder. These subjects suffered their first psychotic episode and were of comparable age to our participants. No information about previous number of non-psychotic episodes of mood disorders was provided [17]. In general when relevant information was provided, the previous positive studies among familial patients found abnormalities only in older patients with longer duration of illness and a greater severity of illness as manifested by presence of psychosis.

When investigating the above-mentioned potential sources of SGC volume abnormalities, we found no association between total SGC volumes and length of illness expressed by duration of illness, duration of treatment, number of episodes, number of manic episodes. This is not surprising considering the truncated, narrow range of values caused by the fact, that we recruited young subjects at the early stages of the illness. Also there were no differences between patients with versus without personal history of psychotic symptoms during episodes of mood dysregulation, however due to small number of such patients, these analyses were underpowered.

Our affected group, which consisted of outpatients, was similar to familial mostly euthymic subjects, with comparable duration of illness to our patients from studies by Soares and colleagues [2, 26], which also found comparable SGC volumes between patients and controls. The present study is virtually identical in design to our previous study from an independent, completely unrelated sample of relatives of bipolar patients and controls [12]. There is no overlap in patients between these two studies. The affected subjects included in this study had a slightly higher burden of illness than subjects in our previous investigation, as evidenced by greater percentage of medicated subjects, greater percentage of bipolar patients (60 vs. 30%) in this versus our previous study. Having already experienced manic or hypomanic episodes, more subjects included in this second study belonged to a more progressed stage in the development of bipolar illness. It is encouraging from the clinical perspective that even these more medicated subjects with greater proportion of subjects with personal history of mania/hypomania also had comparable SGC volumes to healthy controls and unaffected relatives. It thus appears that initial manic episodes (mean 1.1, SD = 1.9) do not exert toxic effects on the brain. This is in keeping with our metaanalysis, where actually unipolar subjects showed greater SGC volume decreases than bipolar patients [15].

We also found comparable volumes of SGC between sporadic bipolar patients and healthy controls. This is in keeping with all other previous studies in sporadic bipolar [2, 17, 27], but also unipolar subjects [2]. Since previous

comparisons of sporadic and control subjects showed small effect sizes, we combined data from all previous studies of sporadic patients with mood disorders and this comparison also yielded comparable volumes of SGC between sporadic patients and healthy controls and very low, clinically or biologically insignificant effect sizes (<0.2).

There are several limitations of this study. A prospective design would better allow us to capture changes in neuroanatomy related to neurodevelopment. Clinical heterogeneity may decrease effect sizes and increase risk of type II error [18]. Some of the affected offspring suffered from unipolar depression. This is however most typically the first manifestation of an illness even in patients, who later develop BD [6, 16]. Also about 70% of depressed first-degree relatives of bipolar probands are in fact bipolar [1] and if we want to study early manifestations of bipolar disorders, inclusion of these likely pseudo-unipolar subjects with family history of bipolar disorders is inevitable. Aside from bipolar I subjects, we included probands with conservatively defined bipolar II disorders. There were no volumetric differences between relatives of bipolar I and II subjects. We thus feel that clinical heterogeneity did not affect our data, especially as our patient sample did not contain comorbid conditions, such as ADHD, which may influence brain volumes. The differences between sporadic bipolar and affected offspring of bipolar parents in relevant clinical and demographic variables did not allow for direct comparison of these two groups.

A clear strength of this study lies in the fact that we used hypothesis-driven ROI measurements using previously validated methods and careful controls against experimental bias (blinding of rater, tests of interrater reliability). This study used a HR design, i.e. investigation of affected as well as unaffected offspring of bipolar patients around the age of illness onset. Since participants were included based on clear-cut bipolar disorders in parents, this design allowed us to bypass the diagnostic issues around bipolar disorders in young subjects.

In summary, this study found comparable volumes of SGC among affected, unaffected offspring of bipolar parents and healthy controls. SGC changes were also absent in affected offspring of bipolar parents at the early stages of illness. This is in keeping with the only previous HR design study and further supports the notion that SGC volume abnormalities do not meet criteria for endophenotype. Currently it is not clear at which stage of illness do the SGC volume changes appear and this warrants further investigation. Also similar to previous studies of sporadic patients, we report a lack of SGC volume abnormalities in bipolar patients without family history at the early stages of illness. Even when combining data from all existing studies of sporadic patients, SGC volumes are comparable between these patients and controls.

Acknowledgments This study was supported by grant NR8786, from the Internal Grant Agency of Ministry of Health, Czech Republic. The authors thank Pavla Stopkova, M.D., Ph.D. for help with proband enrollment and Ms. Jolana Sediva for administrative and technical support.

Conflict of interest statement None of the authors has any conflict of interest to disclose.

References

1. Blacker D, Tsuang MT (1993) Unipolar relatives of bipolar pedigrees: are they bipolar? *Psychiatr Genet* 3:5–16
2. Brambilla P, Nicoletti MA, Harenski K, Sassi RB, Mallinger AG, Frank E et al (2002) Anatomical MRI study of subgenual prefrontal cortex in bipolar and unipolar subjects. *Neuropsychopharmacology* 27:792–799
3. Cox RW (1996) AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 29:162–173
4. Drevets WC, Price JL, Simpson JR Jr, Todd RD, Reich T, Vannier M et al (1997) Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 386:824–827
5. Duffy A, Alda M, Crawford L, Milin R, Grof P (2007) The early manifestations of bipolar disorder: a longitudinal prospective study of the offspring of bipolar parents. *Bipolar Disord* 9:828–838
6. Duffy A, Alda M, Kutcher S, Cavazzoni P, Robertson C, Grof E et al (2002) A prospective study of the offspring of bipolar parents responsive and nonresponsive to lithium treatment. *J Clin Psychiatry* 63:1171–1178
7. Duffy A, Hajek T, Alda M, Grof P, Milin R, MacQueen G (2009) Neurocognitive functioning in early stages of bipolar disorder: visual backward masking performance in high risk subjects. *Eur Arch Psychiatry Clin Neurosci* 259(5):263–269
8. Endicott J, Spitzer RL (1978) A diagnostic interview: the schedule for affective disorders and schizophrenia. *Arch Gen Psychiatry* 35:837–844
9. Goldapple K, Segal Z, Garson C, Lau M, Bieling P, Kennedy S et al (2004) Modulation of cortical-limbic pathways in major depression: treatment-specific effects of cognitive behavior therapy. *Arch Gen Psychiatry* 61:34–41
10. Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 160:636–645
11. Hajek T, Carrey N, Alda M (2005) Neuroanatomical abnormalities as risk factors for bipolar disorder. *Bipolar Disord* 7:393–403
12. Hajek T, Gunde E, Bernier D, Slaney C, Propper L, Grof P et al (2007) Subgenual cingulate volumes in affected and unaffected offspring of bipolar parents. *J Affect Disord*
13. Hajek T, Gunde E, Bernier D, Slaney C, Propper L, MacQueen G et al (2008) Pituitary volumes in relatives of bipolar patients: high-risk study. *Eur Arch Psychiatry Clin Neurosci* 258:357–362
14. Hajek T, Gunde E, Slaney C, Propper L, MacQueen G, Duffy A et al (2008) Striatal volumes in affected and unaffected relatives of bipolar patients—high-risk study. *J Psychiatr Res* 43(7): 724–729
15. Hajek T, Kozeny J, Kopecek M, Alda M, Hoschl C (2008) Reduced subgenual cingulate volumes in mood disorders: a meta-analysis. *J Psychiatry Neurosci* 33:91–99
16. Hillegers MH, Reichart CG, Wals M, Verhulst FC, Ormel J, Nolen WA (2005) Five-year prospective outcome of psychopathology in the adolescent offspring of bipolar parents. *Bipolar Disord* 7:344–350
17. Hirayasu Y, Shenton ME, Salisbury DF, Kwon JS, Wible CG, Fischer IA et al (1999) Subgenual cingulate cortex volume in first-episode psychosis. *Am J Psychiatry* 156:1091–1093
18. Kempton MJ, Geddes JR, Ettinger U, Williams SC, Grasby PM (2008) Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder. *Arch Gen Psychiatry* 65:1017–1032
19. Keri S, Kelemen O, Benedek G, Janka Z (2001) Different trait markers for schizophrenia and bipolar disorder: a neurocognitive approach. *Psychol Med* 31:915–922
20. Mayberg HS, Brannan SK, Tekell JL, Silva JA, Mahurin RK, McGinnis S et al (2000) Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. *Biol Psychiatry* 48:830–843
21. Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA et al (1999) Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *Am J Psychiatry* 156:675–682
22. Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C et al (2005) Deep brain stimulation for treatment-resistant depression. *Neuron* 45:651–660
23. McDonald C, Marshall N, Sham PC, Bullmore ET, Schulze K, Chapple B et al (2006) Regional brain morphometry in patients with schizophrenia or bipolar disorder and their unaffected relatives. *Am J Psychiatry* 163:478–487
24. Ongur D, Drevets WC, Price JL (1998) Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci USA* 95:13290–13295
25. Phillips ML, Drevets WC, Rauch SL, Lane R (2003) Neurobiology of emotion perception I: The neural basis of normal emotion perception. *Biol Psychiatry* 54:504–514
26. Sanches M, Sassi RB, Axelson D, Nicoletti M, Brambilla P, Hatch JP et al (2005) Subgenual prefrontal cortex of child and adolescent bipolar patients: a morphometric magnetic resonance imaging study. *Psychiatry Res* 138:43–49
27. Sharma V, Menon R, Carr TJ, Densmore M, Mazmanian D, Williamson PC (2003) An MRI study of subgenual prefrontal cortex in patients with familial and non-familial bipolar I disorder. *J Affect Disord* 77:167–171