Intrapair Differences in Hippocampal Volume in Monozygotic Twins Discordant for the Risk for Anxiety and Depression

Eco J. C. de Geus, Dennis van't Ent, Saskia P.A. Wolfensberger, Peter Heutink, Witte J.G. Hoogendijk, Dorret I. Boomsma, and Dick J. Veltman

Background: Current biological psychiatric models assume that genetic and environmental risk factors for anxiety and depression act on the same brain structures.

Methods: To test this assumption, we assessed brain anatomy by using optimized voxel-based morphometry on magnetic resonance images obtained in monozygotic twin pairs who were discordant for the risk of anxiety and depression ($n = 10$ pairs) and in monozygotic twin pairs who were concordant for high ($n = 7$ pairs) or low ($n = 15$ pairs) risk for anxiety and depression.

Results: We observed volume reductions in the temporal lobe, most notably in the left posterior hippocampal region in subjects at high risk for anxiety and depression, but exclusively in the intrapair comparison of discordant monozygotic twins. Because monozygotic twins are genetically identical, any discordance in their risk for anxiety and depression and hippocampal volume must arise from differential exposure to environmental influences. A group comparison between pairs concordant for low or high risk, which is more likely to reflect differences in genetic vulnerability, did not show reduced temporal-lobe and posterior hippocampal volumes in the pairs at high risk for anxiety and depression.

Conclusions: This pattern of results suggests that damage to temporal-lobe structures may be specific to an environmentally driven etiology of anxiety and depression.

Key Words: MRI, psychopathology, twin study, voxel-based-morphometry

Anxiety and depressive disorders are known to be caused in part by genetic factors, with heritability estimates fluctuating between 20% and 40% (Hettema *et al.* 2001; Kendler *et al.* 2001; Sullivan *et al.* 2000). To identify the neurobiological pathways that harbor the genetic susceptibility to these disorders, deviant brain structure or function measured by magnetic resonance imaging (MRI) has been proposed as an intermediate phenotype (Hariri *et al.* 2002, 2005; Hariri and Weinberger 2003). These attempts to use brain imaging to unravel the genetic etiology of anxiety and depression have not been matched by similar attempts to unravel the impact of environmental risk factors on the brain. This may be in part because of the heterogeneous nature of environmental risk for psychopathology and the difficulty of standardized measurement of the environment (compared with genotyping). Yet about 60%–80% of the risk for these disorders must be attributed to environmental causes. Some of these may operate independent and additive to genetic predisposition, whereas others may directly interact with genetic predisposition (Caspi *et al.* 2003; Eley *et al.* 2004; Grabe *et al.* 2005; Kaufman *et al.* 2004; Kendler *et al.* 2005).

From the Department of Biological Psychology, Vrije Universiteit Amsterdam (EJCdG, DvE, PH, DIB); and Department of Psychiatry and Radiology, Research Institute Neuroscience, GGZ Buitenamstel, Vrije Universiteit Medical Center (SPAW, WJGH, DJV) and Centre for Neurogenomics and Cognitive Research, Vrije Universiteit Medical Center (EJCdG, PH, WJGH), Amsterdam, The Netherlands.

Address reprint requests to E.J.C. de Geus, Ph.D., Department of Biological Psychology, Vrije Universiteit, van der Boechorststraat 1, 1081 BT, Amsterdam, The Netherlands; E-mail: eco@psy.vu.nl.

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Beyond estimating heritability, the study of twins can be used to establish causal effects of environmental factors on individual trait variation (Hyde *et al.* 1995; Castellanos *et al.* 2003). Because monozygotic (MZ) twins are genetically identical, any discordance in their risk for anxiety and depression must arise from differential exposure to environmental influences. Here, we use voxel-based morphometry (VBM; Ashburner *et al.* 1997; Ashburner and Friston 1999, 2000, 2001) on MRI images of MZ twin pairs that are strongly discordant for the risk for anxiety and depression. This technique compares direct size variations of anatomic structures on a voxel-to-voxel basis. Its application is exceptionally powerful in MZ twins because the error introduced by spatial normalization is minimized in intrapair comparisons by the large resemblance in overall MZ brain volumes, which are highly correlated ($r > .85$; Baare *et al.* 2001; Posthuma *et al.* 2002; Toga and Thompson 2005). The expectation is that the MZ intrapair comparison will highlight brain regions linked to anxiety and depression that are particularly susceptible to environmental factors. On the basis of a large volume of studies, these regions are expected to include medial temporal-lobe structures, in particular the amygdala and hippocampal region (Campbell *et al.* 2004; Geuze *et al.* 2005; Videbech and Ravnkilde 2004).

Current biological psychiatric models implicitly assume that the environmental risk factors for anxiety and depression act along the same neurobiological pathways as the genetic risk factors. In view of the heterogeneous nature of these disorders, we cannot exclude the possibility that some parts of the brain are more affected by genetic risk factors, and others more by environmental ones. To address this question directly, we also included concordant MZ twin pairs in the study in which both members were either at very high or very low risk for anxiety and depression. These two groups of concordant MZ twins are likely to reflect a contrast in genetic vulnerability for anxiety and depression, which was confirmed by a comparison of the

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anxiety, depression, and neuroticism levels in the parents of these two twin groups. If genetic and environmental risk factors affect the brain in a similar way, the volume differences found in intrapair contrasts in the discordant twin pairs should be repeated in the group comparison of concordant high- versus concordant low-risk twin pairs.

Methods and Materials

Participants

In a sample of 3324 twin pairs registered in The Netherlands Twin Registry and aged between 18 and 50 years, an average risk score for anxious depression was computed on the basis of longitudinal survey data on anxiety, depression, neuroticism, and somatic anxiety collected in 1991, 1993, 1997, and 2000 (for details, see Boomsma *et al.* 2000). This risk score was shown to have strong predictive validity for clinical anxiety and clinical depression, as assessed by the Composite Diagnostic Interview (Middeldorp *et al.* 2006).

Discordant MZ twin pairs were considered eligible for participation if both were right-handed, they had risk scores at least .5 SD above or below the mean, and their scores were at least 2 SDs apart. This yielded 31 pairs, of whom we invited 17 pairs because they lived near Amsterdam and had filled out our most recent survey. Two pairs were excluded because one of the members had epilepsy or was pregnant. Four pairs refused to participate, mainly because of time constraints. This left a final 11 MZ pairs who were extremely discordant on the risk for anxiety and depression.

Concordant MZ twin pairs were recruited in a comparable way. Monozygotic twin pairs were considered eligible for participation if both were right-handed and both their scores were at least .8 SD above (concordant high) or below (concordant low) the mean risk score for anxiety and depression. This yielded 115 high-concordant and 137 low-concordant pairs, of whom we invited the 48 pairs who lived near Amsterdam and had filled out our most recent survey. Five pairs were excluded because one of the members had a medical illness or was pregnant. Twenty-one pairs refrained from participating, mainly out of time constraints. This left a final 15 MZ pairs who were concordant for a low risk for anxiety and depression and 7 MZ pairs who were concordant for a high risk for anxiety and depression.

During the selection procedure, twins were classified as MZ by using survey items on physical similarities and frequency of confusion by family members. During the actual MRI study, zygosity was established by typing all subjects on 11 DNA microsatellite markers. One discordant pair was dizygotic (DZ) rather than MZ. Because the monozygosity is crucial in the pairwise comparisons, this pair was excluded, leaving 10 MZ-discordant pairs. Two concordant-low twin pairs also were DZ. Although the group comparison of concordant-high and -low pairs does not depend on zygosity, we repeated that analysis with and without these pairs.

Of the final remaining group of 31 MZ twin pairs, the mean age was 29.5 years (range, 20–42 y; 14 male, 17 female). The ethical review board of the Vrije Universiteit Medical Center approved the study, and all participants provided written informed consent.

Survey Data on Lifestyle and Demographics

Around the time of participation in the study, all subjects filled out a detailed survey on lifestyle and demographics, from which we extracted the degree of urbanization of the subjects' current domicile, educational attainment, mean neighborhood income, and presence and stability of a partner relationship. From the survey, we further extracted religious practices, exercise participation, alcohol use, smoking behavior, and current somatic health (asthma, rheumatoid arthritis, or allergy; urinogenital or kidney disease; skeletomuscular problems; migraine or Parkinson's disease; hypertension, diabetes, or cardiovascular disease; neoplasms) and the use of medication. Finally, subjects were requested to obtain their birth weights from a reliable source like maternal report, birth cards or tiles, or hospital records.

Parental Data

Parents of the twins received the same surveys as the twins in 1991, 1993, 1997, and 2000. A score for anxiety and depression for 18 fathers and 21 mothers of the participating twin pairs was obtained in the same way as for the twins, although the Beck Depression Inventory (BDI), rather than the depression subscale of the Young Adult Self-Report scale, was used to assess depression (for details, see Boomsma *et al.* 2000). Furthermore, from the parental surveys, information on birth order, adverse prenatal behaviors, duration of pregnancy, obstetric complications, incubation time (if any), duration of breast feeding, and early head trauma were obtained for 24 of the twin pairs. These mothers also reported on whether the twins had been separated for a prolonged period of time in their youth.

Procedure

Subjects came to the MRI scan unit, where the experimental procedures were first explained in detail. Twins were randomly assigned to an MRI-scan session or to a psychometric session, each of which lasted about 90 min. After completion of the first session, the twins switched sessions. In the psychometric session, cognitive abilities and current affective state were assessed. Each subject was interviewed by using the Composite International Diagnostic Interview (Peters and Andrews 1995; Wittchen 1994) to establish current psychiatric diagnosis over the last year. In addition, the Montgomery-Asberg Depression Rating Scale (MADRS) and the BDI were used to assess depressive symptom characteristics and severity scores (Beck *et al.* 1961; Montgomery and Asberg 1979). Furthermore, the State-Trait Anxiety Inventory (Spielberger 1970) was administered before and after MR scanning.

Estimates of intelligence quotient (IQ) were obtained by using the verbal comprehension scale from the Groningen Intelligence Test (GIT; Luteijn and van der Ploeg 1983) and two working-memory scales (forward and backward digit span) from the Wechsler Adult Intelligence Scale (Wechsler 1997). Finally, social support was measured by using the Duke–UNC questionnaire (Broadhead *et al.* 1988), and subjects were asked to indicate whether they had gone through 21 major life events. These were listed on paper and included individual events (e.g., maltreatment, disease, financial problems, job strain, relational problems) as well as network-related events (e.g., disease or loss of close kin). Subjects were asked to indicate these events in four temporal categories, that is, whether they occurred during the last 6 months, between 6 and 12 months ago, between 1 to 5 years ago, or more than 5 years ago. For each event, they indicated the impact on their lives on a 10-point visual analog scale ranging from no impact to extreme impact.

The MRI session consisted of a structural part of 7 min enclosed in 35 min of functional MRI. The MR imaging of the brain was performed on a 1.5-T Sonata MR system (Siemens, Erlangen, Germany) with a standard circularly polarized head

coil. For anatomic scanning, we used a coronal 3-D gradient-echo T1-weighted sequence (magnetization prepared rapid gradient echo [MPRAGE]; sinversion time: 300 msec, time to repetition (TR) = 15 msec; echo time (TE) = 7 msec; flip angle = 8°; 160 slices, 1 × 1 × 1.5 mm voxel size). Gray-level resolution was 16 bit.

At the end of both sessions, subjects were debriefed and received sets of buccal swabs to collect mucosal cells for DNA extraction.

Volumetric Analysis

Before volumetric analyses, the integrity of the acquired MR images was visually checked, and the origin of each MRI volume was aligned on the anterior-commissure landmark. Differences in brain anatomy were assessed on a voxel-by-voxel basis by using the optimized VBM method proposed by Good et al (2001), an extension of the originally introduced standard VBM technique (Ashburner and Friston 2000, 2001; Wright *et al.* 1995). The automated procedures of VBM were implemented by using the MATLAB (The MathWorks, Inc, Natick, Massachusetts) VBM tools that were developed by Christian Gaser from the University of Jena, Germany, based on original scripts by John Ashburner and SPM2 analysis software (Department of Imaging Neuroscience, Wellcome, London, United Kingdom).

In a first step, standard VBM is used to create study-specific T1 MRI templates for the whole brain and customized gray-matter (GM), white-matter (WM), and cerebrospinal fluid (CSF) priors. To this end, each raw T1 MRI image was spatially normalized to a standard T1 template that is available in SPM2 (Ashburner *et al.* 1997; Ashburner and Friston 1999). Subsequently, the three brain compartments were extracted after image segmentation by using voxel-by-voxel probability mapping with respect to standard GM, WM, and CSF priors as available in SPM2. Each volume was spatially low-pass filtered by using an 8-mm full width at half-maximum (FWHM) isotropic Gaussian kernel. Finally, individual study-specific T1 templates and GM, WM, and CSF priors were created for the discordants and concordants by averaging the computed volumes separately across the twins in the respective groups.

In a following step, the raw MRI images were segmented. Compared with standard VBM, the optimized protocol by Good et al (2001) includes a number of additional processing steps aimed at reducing the probability of voxel-classification errors. In this study, we were interested primarily in regional changes of GM. First, the GM partition in native, not normalized, space was extracted from the raw T1 images by using the newly obtained customized whole-brain T1 templates and GM priors. Subsequently, the spatial normalization parameters for the GM compartment with respect to the GM template were estimated. The obtained deformation matrix–parameter set then was applied to the raw whole-brain MR images. Subsequently, the whole-brain T1 image was resegmented by using parametric mapping with respect to the customized priors, and the normalized GM was extracted. The resolution of the extracted GM images was 1 mm^3. During segmentation, the images were corrected for intensity nonuniformities that were introduced by the MR scanner.

To preserve volumetric information in the normalized images, a modulation step was added by multiplying each voxel-intensity value by the determinant of the Jacobian matrix defining the spatial transformation from the original MR images to each individual GM template. In this way, intensity values of voxels belonging to brain structures expanded during the warp are reduced, whereas voxel intensities from brain structures that are contracted are increased. As a result, voxel-intensity values code for differences in regional brain size that are present in the raw MR images. Before statistical analyses, the modulated images were smoothed by using a 12-mm FWHM isotropic Gaussian kernel to ensure that the image data correspond better with the Gaussian statistics underlying the statistical parametric-mapping technique to detect morphologic differences (Worsley *et al.* 1996). Spatial smoothing also renders the data more normally distributed and reduces the influence of inaccuracies in spatial normalization of individual brains on the following morphometric comparisons.

Statistical Analysis

Differences in the survey- and interview-based variables were tested by a mixed-model analysis of variance (ANOVA; general linear model [GLM] menu item in SPSS; SPSS, Chicago, Illinois) with type of twin pair (discordant, concordant low, concordant high) and risk-score level (high, low) as two fixed factors and with family as a random factor to account for within-family dependence. Primary planned contrasts were the comparison of the low-risk versus the high-risk twin within the discordant pairs, and of the concordant high-risk versus the concordant low-risk pairs. An alpha of .05 was chosen for these tests. Differences in the overall GM volumes between the three groups (discordant, concordant low, concordant high) were tested by using a similar mixed-model ANOVA.

Differences in regional brain volumes as a function of the risk for anxiety and depression were assessed by means of voxel-by-voxel parametric mapping by using a paired *t* test (discordant pairs) or a one-way ANOVA test (concordant groups) in SPM2. Statistical tests were performed on the modulated GM compartments with, in each test, global GM volume included as a covariate to focus on regional volume changes that were disproportionate with respect to overall GM size. In each comparison, two-tailed difference contrasts were applied testing for possible regional volume increases or decreases of brain structures in one group compared with the other. In a primary analysis, volumetric changes were considered significant at a p value of .05, corrected for multiple testing. Because we had a relatively small number of twins, in a secondary analysis, this criterion was relaxed to an uncorrected $p < .001$, with a minimum cluster size of 50 contiguous voxels meeting this p value.

Results

Table 1 displays the mean anxious-depression, (somatic) anxiety, and neuroticism scores for the entire twin sample (column 1) and for the MZ twins who were selected to be at low or high risk for anxiety and depression. Mean scores are given across all of the four possibly available surveys. Significant differences were found between the low- and high-risk twins of the discordant pairs on all four variables. Likewise, the low-risk concordant twins significantly differed in the expected direction on all variables from the high-risk concordant twins.

To confirm our expectation that our selection for low-risk versus high-risk concordant twin pairs mainly reflects a genetic contrast, we analyzed parental data for all pairs where this information was available. Table 2 displays the mean scores on depression, (somatic) anxiety, and neuroticism of the twin's parents. Analysis of variance with parents as the repeated factor and with twin type (discordant, concordant low, and concordant high) as the between factor confirmed higher values for all risk traits in the parents of the concordant high-risk twin group than in the parents of the concordant low-risk twins. Parents from

Table 1. Risk for anxiety and depression in the full Netherlands Twin Registry sample and in the selected Concordant and Discordant twin pairs

	All Twins (no selection)	Low Risk Concordant Twins	Discordant Twin pairs		High Risk Concordant Twins	
			Low Risk Twin	High Risk Twin		
	$6298 < N < 6648$	$N=30$	$N=10$		$N=14$	
Anxious Depression	4.9 (4.1)	1.4 (1.2)	2.6 (1.0)	10.4 (4.5)	11.4 (5.0)	[a,b]
Anxiety	33.4 (7.9)	25.4 (3.1)	27.9 (3.5)	46.0 (8.7)	44.8 (8.5)	[a,b]
Somatic Anxiety	18.3 (5.0)	13.4 (1.6)	16.7 (2.9)	24.9 (6.1)	26.6 (7.7)	[a,b]
Neuroticism	51.8 (22.3)	26.8 (11.5)	30.4 (12.4)	68.8 (18.5)	84.9 (15.9)	[a,b,c]

[a]Significant difference between Low risk and High risk concordant pairs.
[b]Significant intrapair difference between the Low risk and High risk twin from discordant pairs.
[c]Significant difference between High risk concordant pairs and the High risk subject from discordant pairs.

discordant pairs did not differ from the parents of the concordant low-risk twins but had significantly lower scores on the BDI and the somatic-anxiety scale than did the parents of high-risk twins. This suggests that the high-risk discordant twin was the so-called odd person out in these families.

Subject characteristics at the time of MRI scanning are shown in Table 3. Age and male–female distribution were not significantly different across the groups. Only one subject in a concordant high-risk pair obtained a current diagnosis of depression. One further subject currently used antidepressant medication (selective serotonin-reuptake inhibitor). This was the subject with the high anxious-depression score from a discordant pair. Mixed ANOVA confirmed that the concordant low-risk pairs scored significantly lower on the MADRS, BDI, and state-anxiety measures than did the concordant high-risk pairs. Within the discordant pairs, the MADRS, BDI, and state-anxiety measures all showed significant intrapair differences in the expected direction.

The verbal IQ subscale and the working-memory IQ subscales did not show any significant differences between low- and high-risk twins, either between the concordant groups or within discordant pairs.

Total GM Volume

Before analysis of regional size differences, we set out to investigate whether there was any difference in global non-normalized GM volume between the members of a pair in the discordant twins as well as between concordant low- and high-risk pairs. Figure 1 compares total GM volumes (in milliliters) of each twin pair in the study. Each plotted symbol represents one pair. The position on the horizontal axis represents the brain volume of one twin, and the position on the vertical axis, the volume of the other twin. For both the concordants (open and filled circles) and the discordants (asterisks), the data are scattered around the dashed diagonal, representing identity, indicating that the overall brain sizes of the twins in every pair are highly similar. The latter observation was confirmed by means of paired *t* tests, performed separately for each group, which revealed no significant differences for total brain volumes within each pair. Furthermore, mixed ANOVA yielded no significant group differences in GM volume of the concordant high-risk twins (mean ± SD: 683 ± 54 mL), the concordant low-risk twins (671 ± 54 mL), or the discordant twins (682 ± 43 mL).

Regional Morphometry

Discordant Twin Pairs. Volumetric analyses yielded evidence of significant intrapair differences only after the statistical criteria were relaxed to befit a region-of-interest analysis rather than a whole-brain comparison. Compared with their low-risk co-twin, the high-risk twins showed a decrease of GM volume in multiple areas of the left temporal lobe, including a region at the occipital–temporal border (cluster 1: maximum $t = 10.42$ at x = −42, y = −65, z = −15 in Montreal Neurological Institute [MNI] space), a midtemporal area (cluster 2: maximum $t = 6.28$ at x = −56, y = −34, z = −18), and two regions in the temporal pole (cluster 3: maximum $t = 8.04$ at x = −62, y = −2, z = −5; cluster 4: maximum $t = 6.19$ at x = −55, y = 5, z = −11). These areas are depicted in Figure 2. In addition to these regions, a fifth area of reduced volume was found in the medial temporal lobe that included the left parahippocampal formation extending into parahippocampal gyrus (voxel level: maximum $t = 8.08$ at x = −24, y = −34, z = −6). Figure 3A shows this voxel cluster in the hippocampus projected on a sagittal slice of the GM template of the 20 twins from the discordant pairs. The relative contribution of each twin pair to the regional difference in modulated GM can be appreciated from the plot in Figure 3B. For the most significant

Table 2. Risk for anxiety and depression in the parents of the selected concordant and discordant pairs

	Low Risk Concordant Twins	Discordant Twins	High Risk Concordant Twins	
Mother/Father	8/6	7/7	6/5	
Age	52	55	56	
Depression mean of 2 surveys	2.0 (3.7)	2.4 (2.8)	5.5 (6.0)	[a,b]
Anxiety mean of 4 surveys	27.9 (6.3)	36.9 (7.5)	42.3 (14.8)	[a,b]
Somatic Anxiety mean of 4 surveys	14.7 (2.8)	15.8 (3.1)	20.0 (5.2)	[a,b]
Neuroticism mean of 4 surveys	33.5 (18.9)	48.8 (21.3)	67.5 (27.1)	[a]

[a]Significant difference between parents of Low risk and High risk concordant pairs.
[b]Significant difference between parents of discordant and High risk concordant pairs.

Table 3. Characteristics of the twins at the time of MRI scanning

	Low Risk Concordant Twins	Discordant Twin pairs		High Risk Concordant Twins	
		Low Risk Twin	High Risk Twin		
	N=30	*N*=10	*N*=10	*N*=14	
Male/Female	14/16	4/6	4/6	6/8	-
Age	30.9	30.6	30.6	26.1	-
BDI depression	1.1 (1.5)	2.7 (2.5)	9.7 (10.6)	8.0 (6.0)	*a,c*
MADRAS	0.43 (1.5)	2.1 (2.5)	5.0 (3.9)	5.1 (7.8)	*a,c*
STAI State Anxiety before Scan session	27.7 (5.0)	31.4 (5.3)	37.0 (7.3)	36.7 (9.8)	*a,c*
STAI State Anxiety after Scan session	25.1 (4.7)	28.3 (5.5)	37.1 (8.1)	33.8 (8.4)	*a,b,c*
Verbal IQ subscale	13.1 (2.5)	14.5 (2.2)	14.6 (2.2)	12.9 (3.9)	-
Working Memory IQ subscale	7.6 (1.3)	8.9 (2.4)	8.3 (3.2)	8.6 (1.6)	-

[a]Significant difference between Low risk and High risk concordants.
[b]Significant differerence between High risk subjects from concordant and discordant pairs.
[c]Significant intrapair difference in discordant twins.

voxel, differences in intensity relative to the mean for all 10 twin pairs are shown consecutively, with data for the high- and low-risk twin of each pair at horizontal tick marks "H" and "L", respectively. In 9 of 10 twin pairs, the plot indicates a very close correspondence of the data to the fitted, sawtooth-shaped paired *t* test model shown in thick gray. Figure 3C shows the posterior hippocampal voxel-cluster centered on sagittal, coronal, and axial slices of the average MRI across the 20 discordant twins. To facilitate interpretation of the anatomic location, the observed region is shown in close-up view, with voxel *p* value threshold raised to $p < .01$ (10-fold of the original).

Coronal and axial views of Figure 3C also illustrate anatomic locations of two of the four additional voxel clusters observed in the left temporal lobe (clusters 2 and 3). The orthogonal-slice views also indicate a voxel cluster on the medial side of the hippocampus (labeled by an asterisk), close to the aforementioned posterior hippocampal region. The maximum of this voxel cluster closely borders the CSF compartment. Because VBM results at intersections between GM or WM and CSF can be spurious, interpreting this cluster as additional evidence of hippocampal volume loss must be performed with caution.

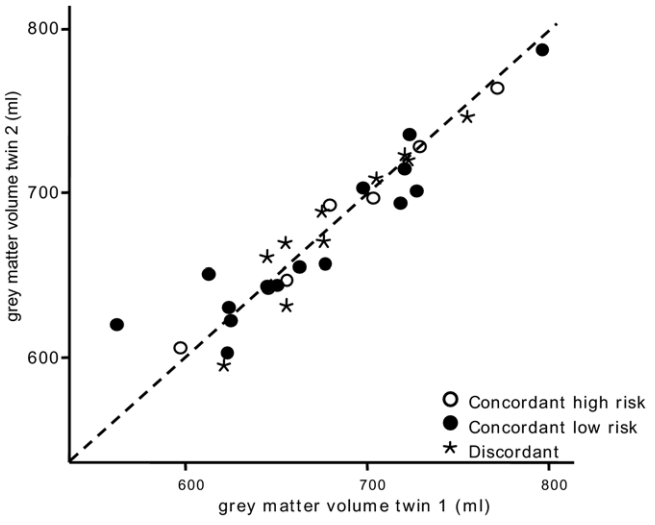

Figure 1. Global gray-matter volumes (in mL) of individual twin pairs. Every symbol corresponds to one pair, with brain size of one twin on the horizontal and brain size of the other twin on the vertical axis. The dashed vertical line represents the identity line (gray-matter volume of twin 1 = gray-matter volume of twin 2). Different symbols denote the individuals from concordant high-risk pairs (open circles), concordant low-risk pairs (closed circles), and discordant pairs (asterisks). In the groups of concordant pairs, the twins were randomly assigned as twin 1 and twin 2. In the discordants, the low-risk twin was assigned as twin 1, and the high-risk twin was assigned as twin 2.

The observed volume reductions in the high-risk twins clearly were unilateral. Post hoc testing at more liberal *p* values did not reveal similar volume reductions in the right temporal lobe, including the right posterior hippocampal region, even when the probability threshold for individual voxels was lowered to $p < .05$.

A single area was found when testing for the reverse contrast, that is, that local GM volume was increased in the high-risk, compared with the low-risk, twins. In the left posterior cingulated, a larger volume was found in the high-risk twins (maximum $t = 9.98$ at $x = -7$, $y = -54$, $z = 32$).

Concordant Low- Versus Concordant High-risk Groups. When comparing the modulated GM extractions of the concordant high-risk group with the compartments of the concordant low-risk group, we found no significant regional volumetric changes in the ANOVA test results. Local GM differences were absent, either when testing for volume decreases in the high- compared with the low-risk group or when testing the opposite contrast, comprising a search for relative volume increases in the concordants for high risk. Even after applying more liberal *p* value thresholds of the statistical parametric maps, we still did not find any indication of GM-volume differences, most notably also not in the left hippocampal region that had been most significant in the comparison of discordant twins. Repetition of the analyses excluding the two pairs who turned out to be DZ still yielded no differences between subjects at low or high genetic risk for anxiety and depression.

Because intrapair *t* testing is more sensitive than between-pair ANOVA comparisons, we repeated the concordant low- versus concordant high-risk comparison in a within-subject design. Each high-risk concordant twin pair was matched with a low-risk concordant pair on the basis of the similarity between the pairs in total GM volumes. This matching recreates some of the advantages of the *t* tests by keeping the variance in the required warping to standard MNI space at a minimum. However, this two (twins within a concordant pair) by two (high risk, low risk) repeated-measures ANOVA did not yield evidence for smaller GM volumes in the concordant high-risk twins, even at liberal *p* value thresholds.

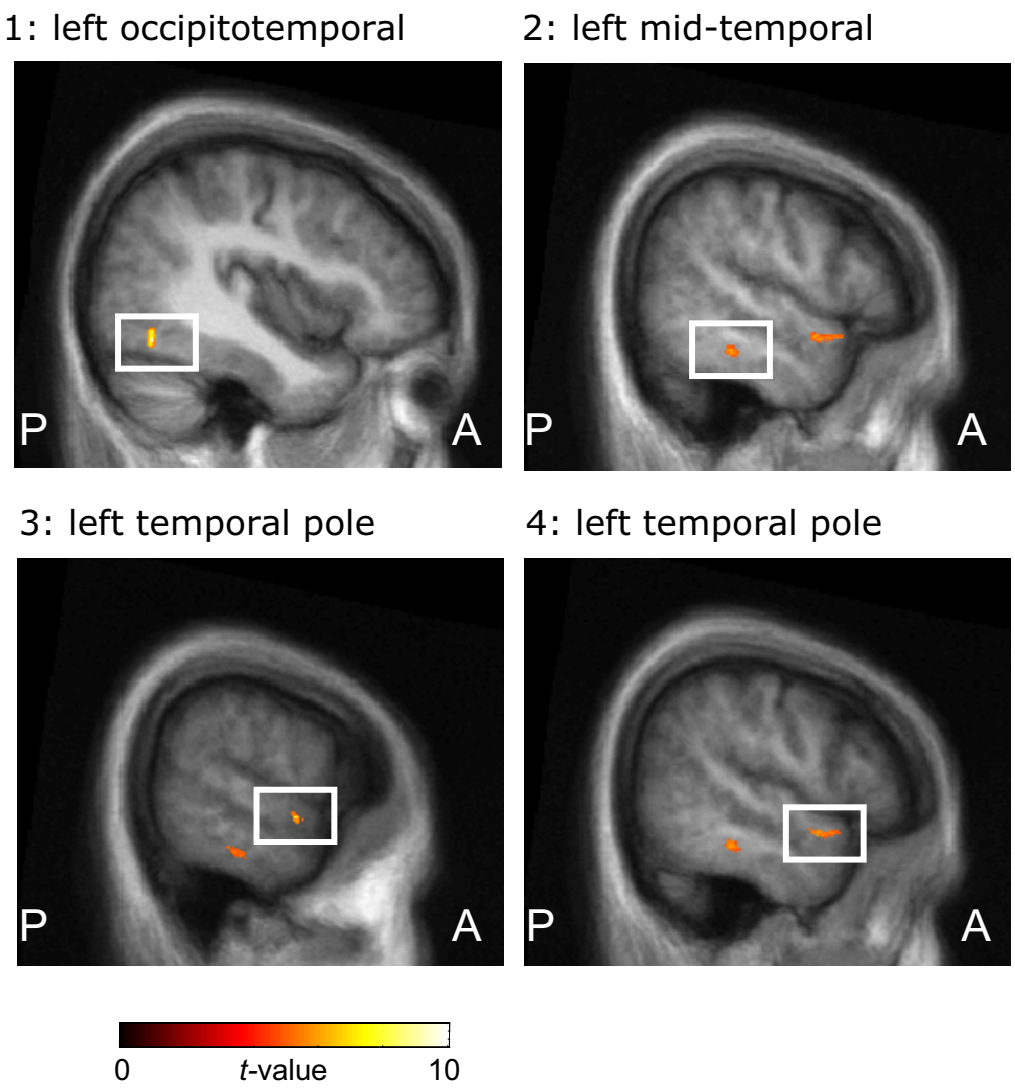

Figure 2. Parametric *t* map of regional gray-matter size reductions in the high-risk, relative to the low-risk, discordant twins. *T* values are mapped by the color bar and projected on the average MR sections of the 20 discordant twins. Four of the five temporal regions that met our criterion of significant volume reduction in the high-risk compared with the low-risk twin ($p < .001$; min, 50 voxels) are shown. A fifth region is shown separately in Figure 3.

Environmental Risk Factors

To explain the discordance in the risk for anxiety and depression in genetically identical subjects, we tested the discordant pairs ($n = 10$) on a number of potential environmental risk factors, which are listed in full in Table 4. In view of the small sample size, the data presented in this table should be considered explorative only. On inspection of the table, two remarkable differences in the low- and high-risk members of the discordant pairs stand out. Significantly lower social support was experienced in the high-risk group, and these twins reported more chronic stress from major life events. Specifically, 9 of the 10 high-risk twins reported major life events that occurred more than 5 years ago, and 5 subjects reported multiple events. Intriguingly, the only high-risk twin not reporting these early life events came from the pair with the poorest fit in the VBM analyses (pair 3 in Figure 3B). For the low-risk twins, only four subjects reported such events, and only one subject reported multiple events. In addition, the average impact of the life events generally was rated to be larger by the high-risk twins.

Additional environmental influences may arise from adverse events or complications during the pregnancies for the discordant twins that may have affected the high-risk discordant twin more than the low-risk discordant twin. To investigate this possibility, we compared prenatal and perinatal events of the discordant twins with those of the concordant high- and low-risk twins. There were no differences in parental age, socioeconomic status, or smoking behavior during pregnancy, but mothers of discordant twins used more medication and consumed alcohol more often. There were no differences in the frequency of incubation, methods of delivery, or birth weight of the concordant and discordant twins. Pregnancy duration was on average 3 weeks longer in the discordant twin pairs than in the concordant twins.

Discussion

We used VBM on MRI images of MZ twin pairs strongly discordant for the risk for anxiety and depression to establish

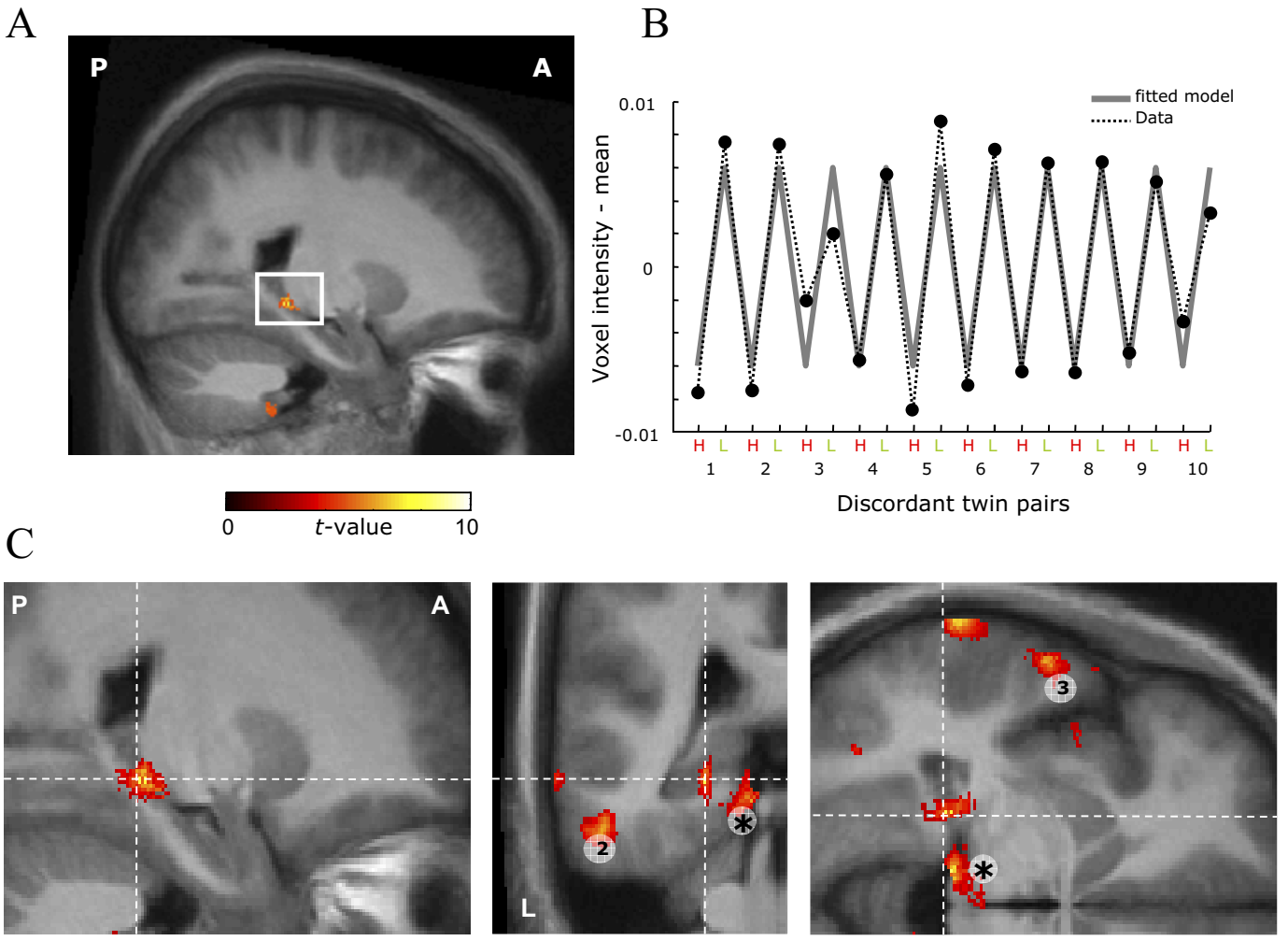

Figure 3. (A) Boxed region contains a left parahippocampal area in which a significant volume reduction was found in the high-risk twin compared within the low-risk twin ($p < .001$; min, 50 voxels). **(B)** Relative responses of the discordant twin pairs at the most significant voxel ($t = 8.08$ at x = −24, y = −34, z = −6 in MNI space). Differences in intensity relative to the mean for all 10 twin pairs are shown as filled circles, connected by a dashed line. From left to right, data for the high-risk and low-risk twin of each pair repetitively at horizontal ticks H and L, respectively. The fitted paired *t* test model is shown in thick gray. **(C)** Parametric *t* map of regional gray-matter size reductions in sagittal, coronal, and axial views of the area boxed in **A** at lower significance ($p < .01$; min, 50 voxels). The cross-haired cursor is at the most significant voxel of the cluster in the parahippocampal region. Coronal and axial views show two additional voxel clusters, 2 and 3, observed in the left temporal lobe (cluster 2: maximum $t = 6.28$ at x = −56, y = −34, z = −18; cluster 3: maximum $t = 8.36$ at x = −62, y = −2, z = −5). *A cluster bordering CSF on the medial side of the parahippocampus (maximum $t = 8.04$ at x = −9, y = −32, z = −6).

intrapair differences in regional brain volumes. This risk was computed on the basis of longitudinal survey data on anxiety, depression, neuroticism, and somatic anxiety collected in 1991, 1993, 1997 and 2000 (for details, see Boomsma *et al.* 2000), which were shown to have strong predictive validity for clinical anxiety and clinical depression, as assessed by the Composite Diagnostic Interview (Middeldorp *et al.* 2006). Because MZ twins are considered genetically identical, any discordance in their risk for anxiety and depression must arise from differential exposure to environmental influences. Hence, we anticipated that the intrapair comparison would highlight brain regions involved in anxiety and depression that are particularly susceptible to environmental factors. Our results place these regions mainly in the left temporal lobe. Most notable were the lower GM volumes in the left posterior hippocampus of the high-risk twins. This region contains the main afferent and efferent connections of the hippocampus to the rest of the temporal lobe (Lavenex and Amaral 2000) and may well constitute the primary deficit.

It should be noted that the number of discordant twin pairs in this study was only modest. A number of smaller regional differences elsewhere in the brain may have gone undetected. To reduce the risk of false negatives, we used a lenient correction for multiple testing in the whole brain comparisons. This, of course, increased the risk of false positives, but our confidence in the temporal-lobe findings is strengthened by comparison to previous studies. Results from our VBM approach converge rather well with those obtained by neuroimaging by using anatomic GM segmentation of medial temporal-lobe structures. Reduced hippocampal volume often has been reported in major depression (Campbell *et al.* 2004; Geuze *et al.* 2005; Videbech and Ravnkilde 2004), even in fully remitted unmedicated patients (Neumeister *et al.* 2005), and smaller hippocampal volumes were predictive of a poor response to antidepressant medication (Hsieh *et al.* 2002; Vakili *et al.* 2000). Reduced hippocampal volumes also have been found in severe trauma-exposed subjects with unremitting posttraumatic stress disorder (Bremner *et*

Table 4. Environmental Risk Factors in the Low and High Risk Twin from Discordant Pairs

	Low Risk Twin *N*=10	High Risk Twin *N*=10
First born	4	6
Birth weight (grams)	2416 (sd=457)	2413 (sd=285)
# of medical conditions	1.1	1.3
Educational attainment (primary only/ vocational/ high school/ college & university)	0/1/4/5	0/0/6/4
Mean income (Euro/year)	33530,00	32950,00
Urbanization (urban/rural)	3/7	2/8
Religion (church going/belief/no)	1/4/5	2/2/6
Stable partner relation (with/without)	4/6	3/7
Current smoker (yes/no)	2/8	3/6
Alcohol use (> 3 glass/wk)	5	5
Exercise participation (> 4 METhour/wk)	5	7
Social Support	32.1 (3.7)[a]	29.0 (2.9)[a]
Major life events		
- last 6 months (events/mean impact)	4/4.3	4/3.2
- 6 to 12 months ago (events/mean impact)	3/2.0	2/3.4
- 1 to 5 years ago (events/mean impact)	6/1.5[a]	6/4.1[a]
- more then 5 years ago (events/mean impact)	4[a]/3.5	9[a]/4.2

[a]Significant intrapair difference in discordant twins (*t*-tests or $\chi 2$, $p < 0.05$).

al. 1995; Freeman *et al.* 1998; Gilbertson *et al.* 2002; Gurvits *et al.* 1996; Vythilingam *et al.* 2002).

The glucocorticoid cascade hypothesis (McEwen and Magarinos 1997, 2001; McEwen 1999, 2001; Sapolsky *et al.* 1986, Sapolsky 2000) provides a powerful neurobiological framework to link differences in environmental risk exposure in discordant MZ twin pairs to differences in their hippocampal volumes. According to this hypothesis, severe life stress may cause a prolonged excess of glucocorticoids that damages the hippocampus. Animal studies as well as observations in patients with Cushing syndrome are in support of toxic effects of high levels of glucocorticoids on the hippocampus (Duman *et al.* 1999; Starkman *et al.* 1992, 1999; Uno *et al.* 1994; Watanabe *et al.* 1992). Because the hippocampal region is a major site of inhibitory control over cortisol release (Herman and Cullinan 1997), this damage may create a vicious circle (or cascade) of increased cortisol levels that further reduce the volume of exactly those hippocampal cells that are needed in negative feedback control over cortisol (McEwen 1999, 2001; McEwen and Magarinos 1997, 2001). Once depressive disorder sets in, the stress inherent in the repeated episodes may itself add to this process, which could explain why the duration of untreated depression is correlated with hippocampal size (Bell-McGinty *et al.* 2002; MacQueen *et al.* 2003; Sheline *et al.* 2003).

We probed our discordant pairs on a number of potential environmental risk factors, including demographic and lifestyle variables and recent major life events and social support structure. Although limited by a potential retrospective bias as a result of their higher negative affect at the time of recall, the high-risk twins reported exposure to more, and more severe, life stressors early in their lives. In keeping with the glucocorticoid-cascade hypothesis, these stressors may well account for part of the environmental effects on temporal GM volume, especially the hippocampal region. However, despite the attractiveness of the glucocorticoid-cascade hypothesis, an alternative environmental factor that could explain the discordance in posterior hippocampal volume and risk for anxiety and depression in our genetically identical twins is epigenetic reprogramming. Epigenetic reprogramming can create large phenotypic divergence in genetically identical subjects by selectively repressing the expression of some genes. Fraga et al (2005) profiled the epigenetic patterns related to global and locus-specific DNA methylation and histone H3 and H4 acetylation in 80 twins across a large age range. Young twins were epigenetically indistinguishable but older twins exhibited remarkable differences in their gene-expression profile which they attributed to the impact of environmental and lifestyle factors. Epigenetic drift, therefore, is a potential source of the discordance in both left-temporal volumes and the risk for anxiety and depression. An important question that needs to be addressed in the future is whether life stress and hypothalamic-pituitary-adrenocortical (HPA)–axis activation can somehow directly impact on epigenetic programming.

In striking contrast to the VBM results in discordant twins, no reduction in GM volume in the hippocampal region was found in the group comparison of concordant low- versus concordant high-risk twins. With our selection of twins concordant for extreme low or high risk, we attempted to select groups that differ strongly in genetic susceptibility. The higher average scores of family members of the high-risk twins compared with family members of the low-risk twins give credence to the idea that these groups indeed represent a genetic contrast. Our results, therefore, suggest that genetic influences on hippocampal volume (or related genes in the HPA axis) may not account for a large part of the heritability of anxiety and depression found in family and twin studies. This does not rule out that such genes have an indirect effect on the risk for anxiety and depression through gene–environment interaction. Genetic influences on hippocampal volume have been found in several studies (Lyons *et al.* 2001; Sullivan *et al.* 2001), and they may well play a causal role in psychopathology by sensitizing the individual to stressful experiences (Gilbertson *et al.* 2002; Gurvits *et al.* 1996).

Basic research has pointed to many systems outside the HPA axis and the hippocampal formation that can harbor susceptibility genes to anxiety and depression (Nestler *et al.* 2002). Most prominent has been the suggestion of genetic defects in serotonergic neurotransmission in both animal (Ansorge *et al.* 2004; Gross *et al.* 2002) and human (Lesch and Gutknecht 2005; Levinson 2006; Sen *et al.* 2004) research. It is important to note that genetic variation in serotonergic neurotransmission appears to directly interact with environmental risk factors, as testified to

by the replicated interaction between early life stress and genetic variation in serotonin neurotransmission (Caspi *et al.* 2003; Eley *et al.* 2004; Grabe *et al.* 2005; Kaufman *et al.* 2004; Kendler *et al.* 2005). This gene–environment interaction could well reflect the effects of stress-induced HPA-axis activation on serotonergic neurotransmission and vice versa. Various neurobiological models for reciprocal influences of the HPA axis on the serotonergic system have been proposed, all of which strikingly converge on the hippocampal formation as the major locus of the interaction (Czeh *et al.* 2001; Jacobs *et al.* 2000; Lopez *et al.* 1998; Roozendaal 2003; Vermetten *et al.* 2003).

In summary, we conclude that the genetic and environmental etiology of mood disorder may differ and that damage to the posterior hippocampal region may be specific to the environmentally driven etiology of anxiety and depression. We reach this conclusion in a design that deliberately selected subjects at so-called pure genetic and pure environmental risk for anxiety and depression. This is unlikely to represent the majority of subjects at risk in the population at large. In most subjects, risk will arise through a combination of genetic and environmental factors. This may explain the heterogeneity of previous studies that have used structural MRI in depression. Although meta-analyses consistently point to a smaller hippocampal volume when all studies are considered jointly, various null findings have been reported in well-designed studies (MacQueen *et al.* 2003; Posener 2003; Vakili 2000). These null findings have been attributed to low disease burden (Campbell *et al.* 2004) because patients in these studies were relatively young. Our discordant MZ twins, however, also were young and, with a single exception, not clinically depressed at the time of MRI scanning. Despite this, a significant reduction in hippocampal volume was found. Thus, we alternatively suggest that studies with null findings may have included relatively fewer subjects with environmental risk factors and more subjects at genetic risk. In support of this idea, a smaller left hippocampal volume in adult women with major depressive disorder was observed exclusively in those who had a history of severe and prolonged physical or sexual abuse in childhood (Vythilingam *et al.* 2002).

In future MRI studies on anxiety and depression, investigators should aim to avoid admixture of subjects who are at risk as a result of genetic factors with those who are at risk as a result of environmental factors.

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