Functional, structural, and molecular imaging of the risk for anxiety and depression S.P.A.Wolfensberger

Functional, structural, and molecular imaging of the risk for anxiety and depression

S.P.A.Wolfensberger

Department of Psychiatry

VU University *Amsterdam*

Functional, structural, and molecular imaging of the risk for anxiety and depression

S.P.A.Wolfensberger

Publication of this thesis has been accomplished with gratefully acknowledged financial support provided by: Department of Psychiatry, VU University Medical Centre, Amsterdam, Janssen Research & Development, Neuroscience, Philips Healthcare Benelux, BV Cyclotron VU and Stichting Ina Veenstra-Rademaker.

VU University Amsterdam

The studies described in this thesis were carried out at the Department of Radiology, Nuclear Medicine and PET research, Psychiatry and Biological Psychology (VU University Medical Centre, Amsterdam). The studies were financially supported by the Netherlands Organization for Scientific Research (NWO) grants 900-562-137, 904-61-090, 98510-002, 904-61-193, 480-04-004, and 575-25-006, the Centre for Neurogenomics and Cognitive Research (CNCR), the Centre for Medical Systems Biology (CMSB), a center of excellence approved by the Netherlands Genomics Initiative/NOW and Janssen Research & Development, Neuroscience.

ISBN 978-90-86595-36-5

Cover design/illustration and lay-out: Esther Beekman
(www.estherontwerpt.nl/www.ideesther.nl)
Printed by: Ipskamp Drukkers BV, Enschede, The Netherlands

VRIJE UNIVERSITEIT

Functional, structural, and molecular imaging of the risk for anxiety and depression

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
op woensdag 11 mei 2011 om 15.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Saskia Pauline Anemoon Wolfensberger

geboren te Amsterdam

promotoren:	prof.dr. W.J.G. Hoogendijk
	prof.dr. A.A. Lammertsma
	prof.dr. J.C.N. de Geus
copromotor:	prof.dr. D.J. Veltman

TABLE OF CONTENTS

1

General introduction and outline

Background

Throughout the course of life, from conception to death, the human body in general and the brain in particular are shaped by (interactive) effects of genes and environment. As a consequence, individual differences in complex traits, including behavioural traits, reflect a mix of variation in genetic make-up and each individual's unique history of exposures to risk and protective environments. This dual contribution of genetic and environmental variance is also prominently visible in psychopathological traits, like anxiety and depressive disorders, which are the focus of the present thesis.

Studies in genetically informative samples, many of which have used a twin design, show that about 40% of clinical anxiety and depression is due to heritable factors, whereas the remaining 60% of the risk for anxiety and depressive disorders can be attributed to environmental factors[1,2], including those that interact with genetic factors[3-7]. A core assumption in this thesis is that these genetic and environmental risk factors primarily act through the brain; by changing the brain at a molecular level, they affect structure and functioning of brain modules that are critical to mood/emotion regulation. This assumption is generally accepted in psychiatry, where depression and anxiety are now regarded to be brain diseases. The present thesis, however, deviates from the 'common view in the field with regard to another implicit assumption, namely *that environmental risk factors have the same pathogenic effects as genetic risk factors*. Instead, it is hypothesized that these two types of risk may have an impact on different brain structures, or that they may affect the same brain structures, but in entirely different, if not opposite, ways.

Approach

To test this hypothesis, structural and functional brain imaging techniques were used in a unique design involving monozygotic (MZ) discordant/concordant twins, which allowed for separation of the effects of genetic and environmental risk factors. In this design, brain imaging was first compared in MZ twin pairs that were strongly discordant for the risk of, for example, generalized anxiety, obsessive compulsive behaviour, or major depression. In these pairs, one twin scored very high on symptoms of these disorders, whereas the co-twin scored very low. As monozygotic twins always are (nearly) 100% identical at the DNA sequence level[8], any discordance at the phenotype level should be the result of differential exposure to environmental influences, such as the lack of social support (environmental deprivation) and life events. Therefore, in these twins, differences in brain activation linked to these behavioural traits also reflect environmental effects on the brain, including epigenetic effects, rather than effects of variation in DNA sequence.

Next, MZ twin pairs were selected that were highly concordant for the risk of these traits. In one group the risk was very high in both twins, whilst in the other group it was very low.

MZ twins can be regarded as repeated sampling of the same genotype that is exposed to partly different environments. If the phenotypic resemblance in both twins is very high, genetic factors are a likely cause of this resemblance. This is particularly true in anxiety and depression, where the only alternative source of resemblance between MZ twins - common environmental factors including the sharing of parents - has been shown to have no significant impact[2]. Comparison of MZ twins that are very high on anxious depressive symptoms with twins that are very low on anxious depressive symptoms, therefore, constitutes a contrast of low versus high genetic risk for anxiety and depressive disorder. Verification of the contribution of genetic factors to the phenotypic contrast between concordant low and concordant high MZ pairs can be done by testing other family members of the twins (e.g. parents or siblings), who are expected to also score very low or very high respectively on anxious depressive symptoms. Differences in brain activation linked to these behavioural traits between twins from the high-risk families and those from the low-risk families should mainly reflect effects of variation in DNA sequences between the two groups.

To date, the above type of imaging genetics is increasingly used[9-18]. In addition, there are many studies that employ a candidate gene approach to specifically look at cerebral effects of potential genetic risk factors. In the latter approach a genetic association is tested between measured variation in a candidate gene, usually obtained by previous association to a psychiatric outcome, and variation in brain structure and function, typically assessed by structural and functional magnetic resonance imaging[19-39]. This approach allows for mapping of the effects of risk genes on neurobiological parameters using neuroimaging, and has the advantage of measures causally closer to genes and gene expression than behaviour. It also provides a means to uncover the neurobiological mechanisms by which gene variants impact on the brain. It has been predicted that this booming field of imaging genetics will see further growth over the coming decade[40-42]. Remarkably, to date most imaging genetics studies have employed electroencephalography, magneto-encephalography, structural MRI, or functional magnetic resonance imaging (fMRI). At present, there has not been a single molecular imaging study using positron emission tomography in which such a monozygotic discordant/concordant twin design has been used in the context of anxiety and depressive disorders. This lack of molecular imaging studies is somewhat puzzling, but may in part be due to the paucity of relevant, adequately evaluated and valid radiotracers needed to specifically investigate the molecular basis of anxiety and depression. PET, however, is the most direct and sensitive method to measure receptor function *in vivo*. Therefore, it should be an ideal method to detect genetic differences in regional density and affinity of receptors for neurotransmitters involved in psychiatric disease.

Aims

The aims of this thesis are twofold. Firstly, using the discordant/concordant twin design, the hypothesis will be tested that genetic and environmental risk factors impact on partly different brain structures or, when they converge on the same brain structures, that they may do so in entirely different, perhaps even opposite, ways. Secondly, a new PET ligand, [^{11}C]R116301, for *in vivo* investigation of NK1 receptors will be evaluated, as it is suspected that this receptor is involved in anxiety and depression. Molecular imaging studies using PET could be the basis of future (genetic) imaging studies focused on pathways involved in anxiety and depression.

Together, functional, structural and molecular approaches promise to advance understanding of neuropsychiatric disorders, in particular anxiety and depression. This is an important mission, because depression and anxiety disorders have high lifetime prevalence (16 and 10%, respectively) and are associated with substantial morbidity and mortality. Unravelling the effects of genetic and environmental risk factors on brain structure and function may be the key to better understanding of the neurobiology of neuropsychiatric disorders. Ultimately, this may lead towards possibilities for early identification of individuals at risk, thereby creating a window for developing preventive strategies, based on individual risk profiles.

Brain imaging techniques in this thesis

Imaging techniques such as fMRI and structural MRI, magnetic resonance spectroscopy (MRS), PET, single photon emission computed tomography (SPECT), provide useful methods to obtain insight in the living brain. With these imaging methods, information can be obtained on neurobiological parameters such as neuronal activation in time and place, volumetry of brain structures, receptor density and affinity, and neurotransmitter concentration. This thesis focuses on structural MRI, fMRI and PET methods, each of which will be briefly introduced below.

Structural MRI

MRI is a relatively new technology, which has been in use for a little more than 30 years. It is a non-invasive imaging technique that is used in clinical practice to acquire high-resolution digital images of water-containing human tissues. It provides detailed and high contrast images everywhere in the body, providing clear distinction between different soft tissues, thereby making it especially useful for brain imaging. MRI uses a powerful magnetic field to align the nuclear magnetization vectors of atomic nuclei (of hydrogen atoms) in water in the body. Absorption and emission of radiofrequency radiation of hydrogen nuclei are detectable by the scanner. This signal can be manipulated by additional magnetic fields

(phase and frequency encoding) to build up enough information to construct an image of the body.

The advance of volumetric measurements of brain structures using structural MRI has resulted in many studies reporting brain structure abnormalities in a number of neuropsychiatric disorders. The hippocampal region/formation is one of the brain structures that has been a focus of research and this has revealed a number of structural abnormalities in a variety of neurological and psychiatric disorders, such as Alzheimer's disease[43,44], mild cognitive impairment[45], schizophrenia[46], major depression[47] and anxiety [48, 9] and others.

MR derived volumetric techniques have demonstrated good validity and reproducibility, and accuracy of the measurements has been shown by MRI volumetric measurement of phantoms with known volumes [50-52]. However, studies on brain structures in neuropsychiatric disorders show inconsistencies, both in terms of laterality (left or right), and in direction of volume changes (increase of decrease). Part of these discrepancies may be due to the different methods used for measuring brain structure.

Voxel based morphometry (VBM)[53] is an automatic whole brain method and is increasingly being used as a tool to examine volume changes at a voxel level, obviating manual volumetry, i.e subjective interpretation of anatomic variations. However, it does not provide absolute volumes of brain structures. Most (pre)processing and analysis is automated in software packages, such as statistical parametric mapping (SPM) (www.fil.ion.ucl.ac.uk/spm/), although many methodological questions remain, including what template to use for normalisation, what level and type of correction to use, and how best to display results.

Functional MRI

Functional magnetic resonance imaging (fMRI) is an extension of traditional MRI. It is one of the most recent neuroimaging modalities. Since the early 1990s, fMRI has increasingly been used to study brain function. It uses the varying magnetic properties of the oxygen transporter molecule haemoglobin (the blood oxygen level dependent (BOLD) effect) to examine brain function in human subjects[54]. Activated nerve cells consume oxygen carried by hemoglobin in red blood cells from local capillaries. The local response to this use of oxygen is an increase in blood flow to regions with increased neural activity (hemodynamic response). The vascular system actually overcompensates for this oxygen need of neurons in an activated state. This leads to a locally increased ratio of oxygenated hemoglobin relative to deoxygenated hemoglobin. Because deoxygenated hemoglobin attenuates the MR signal, the vascular response leads to a signal increase that is related to neural activity.

In most fMRI studies, subjects are scanned while performing a certain task (or 'paradigm'). In such paradigms, conditions of interest alternate with reference conditions to produce differences in BOLD signal intensity. Task conditions have to be repeated in order to reduce the effects of noise, enabling the detection of signal changes that correlate significantly with task stimuli.

Advantages of fMRI are its good temporal resolution, allowing for event related analyses according to task performance, relatively low costs, possibility of repeated measurements, and flexible scan duration. Possible limitations of fMRI are its low signal to noise ratio, its relative measures (i.e. measuring differences in signal activation: active state compared to baseline), sensitivity to artefacts, and the fact that the BOLD signal is not a direct measure of neural activity. BOLD signal intensity changes may therefore be difficult to interpret in terms of underlying neurodynamics[55,56].

PET

The first commercial PET camera was built in 1978, 2 years before the advent of MRI. During the following decades, it was increasingly used as a research tool to measure physiologically relevant processes at a molecular level in the living human body and, more recently, in animal models of disease. PET scanning is a non-invasive quantitative imaging technique involving the use of positron emitting radionuclides. Scans are acquired following intravenous injection of a tracer labelled with a radionuclide (called a radioligand in case of neuroreceptor studies) that accumulates in the tissue to be studied, and decays by emission of a positron (anti-electron). After travelling at most a few millimetres, a positron will combine with an electron and the two particles will annihilate almost instantly, resulting in the simultaneous release of two gamma rays (photons) with an energy of 511 keV (equivalent to the mass of positron or electron) into opposite directions. PET imaging is based on the assumption that simultaneous detection of a pair of these gamma rays by two opposing detectors of the PET scanner, is the result of an annihilation event along a straight line in space connecting these two detectors (line of response). Following the registration of these events, counts from all lines of response (sinograms) are reconstructed to provide maps of radioactivity distribution. By performing repeated scans over time, kinetic information (rate of uptake, clearance) can be obtained. Using tracer kinetic modelling, this can be translated into quantitative measures of physiological parameters of interest.

To perform a scan, a short-lived radioactive ligand or tracer is administered. To this end a positron emitting radionuclide is chemically incorporated into a biologically active molecule. Development of radiotracers has proven to be time and money consuming and can be seen as a limiting factor in PET applications. On the other hand, PET technology can be used to trace the biologic pathway of any compound in living humans and many other species, provided it can be radiolabelled with a PET radionuclide.

Several radiotracers have been developed for imaging receptors, neurotransmitter transporters, enzymes and other molecular targets. Due to the short half lives of most

radionuclides, they must be produced using a cyclotron in close proximity to the PET imaging facility. Two commonly used PET radionuclides are [^{11}C] and [^{18}F] with half-lives of 20 and 110 min, respectively. For example, [^{18}F]MPPF is a ligand of the 5-HT$_{1A}$ receptor, [^{11}C]raclopride a ligand of the D_2/D_3 receptor, and [^{18}F]flumazenil a ligand of the benzodiazepine recognition site on the GABA/benzodiazepine receptor complex. Most commonly, [^{18}F]FDG (fluoro-deoxyglucose) is used for measuring glucose metabolism and [^{15}O]H_2O for measuring rCBF (regional cerebral blood flow).

The properties of a PET tracer depend, amongst others, on its metabolism, and its selectivity and affinity for the target to be studied, i.e. in this thesis the NK1 receptor. Another important property of a PET tracer is the level of non-specific binding. A signal due to specific binding might be lost if the level of non-specific binding (background) is too high.

At the beginning of the neuroimaging era, more than 30 years ago, the very first activation studies were performed using [^{18}F]FDG, followed by [^{15}O]H_2O as the method of choice for measuring neuronal activation. Nowadays, fMRI has gradually replaced [^{15}O]H_2O PET for examining the response of the brain to stimuli. Whereas [^{15}O]H_2O PET has better signal to noise ratio (S/R) and does not suffer from signal loss near bone-air transitions, this is outweighed by the higher temporal and spatial resolution of fMRI. Another advantage of fMRI is that it does not require administration of radioactive tracers, thereby enabling multiple repeated measurements within one subject and flexible duration of experiments. However, PET has the unique capability to detect molecular events at picomolar concentrations, compared with micromolar concentrations in MRS.

1.1 Outline of this thesis

Chapter 2 begins by examining emotion processing in relation to the risk for anxiety and depression. In the literature, functional brain imaging studies have reported deviant amygdala responses to emotional stimuli in subjects suffering from anxiety and depressive disorder, but compared to healthy controls both hyperactivity and hypoactivity have been reported. Using fMRI in extremely discordant (at very low or very high risk for anxiety and depression) MZ twins, in chapter 2 the hypothesis is tested that the basis for these discrepant findings lies in different effects of genetic and environmental risk factors on amygdala functioning .

In **chapter 3**, neuronal correlates of emotional processing are examined during an encoding and recognition paradigm using emotionally salient words in the same design and study sample as used in chapter 2.

In **chapter 4**, the second part of this thesis, volumetric differences in brain structure are examined in relation to the risk for anxiety and depression. As current biological psychiatric models assume that genetic and environmental risk factors for anxiety and depression act on the same brain structures, this assumption is tested in this chapter, by applying optimized voxel-based morphometry to magnetic resonance images obtained in the same MZ twin pairs as mentioned in chapters 2 and 3.

In part 3 of this thesis, **chapter 5** and **chapter 6**, the ligand [^{11}C]R116301 is evaluated as a putative novel PET tracer for the quantification of NK1 receptors. In chapter 5 the presence of an NK1 related specific signal is evaluated using a blocking study and in chapter 6 the optimal model for quantifying NK1 receptors using [^{11}C]R116301 is derived.

In **chapter 7** the main findings of this thesis are summarised and discussed and recommendation of future research are given.

References

1. Kendler KS, Prescott CA. A population-based twin study of lifetime major depression in men and women. *Archives of General Psychiatry* 1999; 56:39-44.
2. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: Review and meta-analysis. *American Journal of Psychiatry* 2000; 157:1552-62.
3. Caspi A, Taylor A, Moffitt TE, Plomin R. Neighborhood deprivation affects children's mental health: Environmental risks identified in a genetic design. *Psychological Science* 2000; 11:338-42.
4. Eley TC, Sugden K, Corsico A *et al*. Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Molecular Psychiatry* 2004; 9:908-15.
5. Grabe HJ, Lange M, Wolff B *et al*. Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden. *Molecular Psychiatry* 2005; 10:220-4.
6. Kaufman J, Yang BZ, Douglas-Palumberi H *et al*. Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. *Biological Psychiatry* 2006; 59:673-80.
7. Kendler KS, Heath A, Martin NG, Eaves LJ. Symptoms of Anxiety and Depression in A Volunteer Twin Population - the Etiologic Role of Genetic and Environmental-Factors. *Archives of General Psychiatry* 1986; 43:213-21.
8. Boomsma DI, Beem AL, van den BM *et al*. Netherlands twin family study of anxious depression (NETSAD). *Twin Research* 2000; 3:323-34.
9. Ahveninen J, Jaaskelainen IP, Osipova D *et al*. Inherited auditory-cortical dysfunction in twin pairs discordant for schizophrenia. *Biological Psychiatry* 2006; 60:612-20.
10. Brans RG, van Haren NE, van Baal GC, Schnack HG, Kahn RS, Pol HE. Heritability of changes in brain volume over time in twin pairs discordant for schizophrenia. *Archives of General Psychiatry* 2008; 65:1259-68.
11. den Braber A, van 't Ent D, Blokland GAM *et al*. An fMRI study in monozygotic twins discordant for obsessive-compulsive symptoms. *Biological Psychology* 2008; 79:91-102.
12. Ettinger U, Picchioni M, Landau S *et al*. Magnetic resonance imaging of the thalamus and adhesio interthalamica in twins with schizophrenia. *Archives of General Psychiatry* 2007; 64: 401-9.
13. Posthuma D, Gosso MF, Altink B *et al*. Imaging genetics & cognition: A pilot study involving the CHRM2 and SNAP-25 genes. *Behavior Genetics* 2007; 37:787.
14. Rijsdijk FV, van Haren NE, Picchioni MM *et al*. Brain MRI abnormalities in schizophrenia: same genes or same environment? *Psychological Medicin* 2005; 35:1399-409.
15. Spaniel F, Tintera J, Hajek T *et al*. Language lateralization in monozygotic twins discordant and concordant for schizophrenia. A functional MRI pilot study. *European Psychiatry* 2007; 22:319-22.
16. van 't Ent D, Lehn H, Derks E *et al*. Functional MRI during performance of the stroop task in monozygotic twins concordant or discordant for attention/hyperactivity problems. *Psychophysiology* 2008; 45:S18.
17. van 't Ent D, Lehn H, Derks EM *et al*. A structural MRI study in monozygotic twins concordant or discordant for attention/hyperactivity problems: evidence for genetic and environmental heterogeneity in the developing brain. *Neuroimage* 2007 April 15;35: 1004-20.
18. van der Schol AC, Vonk R, Brans RG er al. Influence of genes and environment on brain voolumes in twin pairs concordant and discordant for bipolar disorder. *Archives of General Psychiatry* 2009; 66:142-51
19. Fisher PM, Meltzer CC, Ziolko SK, Price JC, Hariri AR. Capacity for 5-HT1A-mediated autoregulation predicts amygdala reactivity. *Nature Neuroscience* 2006; 9:1362-3.
20. Bertolino A, Taurisano P, Pisciotta NM *et al*. Genetically determined measures of striatal D2 signaling predict prefrontal activity during working memory performance. *PLoS One* 2010; 5:e9348.
21. Neumann SA, Brown SM, Ferrell RE, Flory JD, Manuck SB, Hariri AR. Human choline transporter gene variation is associated with corticolimbic reactivity and autonomic-cholinergic function. *Biological Psychiatry* 2006; 60:1155-62.
22. Viding E, Williamson DE, Hariri AR. Developmental imaging genetics: Challenges and promises for translational research. *Development and Psychopathology* 2006; 18:877-92.
23. Brown SM, Hariri AR. Neuroimaging studies of serotonin gene polymorphisms: Exploring the interplay of genes, brain, and behavior. *Cognitive Affective & Behavioral Neuroscience* 2006; 6:44-52.
24. Hariri AR, Drabant EM, Weinberger DR. Imaging genetics: Perspectives from studies of genetically driven variation in serotonin function and corticolimbic affective processing. *Biological Psychiatry* 2006; 59:888-97.
25. Hariri AR, Holmes A. Genetics of emotional regulation: the role of the serotonin transporter in neural function. *Trends in Cognitive Sciences* 2006; 10:182-91.
26. Meyer-Lindenberg A, Buckholtz JW, Kolachana B *et al*. Neural mechanisms of genetic risk for impulsivity and violence in humans. *Proceedings of the National Academy of Sciences of the United States of America* 2006; 103:6269-74.
27. Winterer G, Hariri AR, Goldman D, Weinberger DR. Neuroimaging and human genetics. *Neuroimaging, Pt B* 2005;67:325-+.
28. Brown SM, Peet E, Manuck SB *et al*. A regulatory variant of the human tryptophan hydroxylase-2 gene biases amygdala reactivity. *Molecular Psychiatry* 2005; 10:884-8.

29.Bertolino A, Arciero G, Rubino V *et al*. Variation of human amygdala response during threatening stimuli as a function of 5'HTTLPR genotype and personality style. *Biological Psychiatry* 2005; 57:1517-25.
30.Siegle GJ, Hariri AR, Blumberg HP. Examining genetic influences upon frontal and sensory cortico-limbic connectivity in mood-disordered individuals: Can we determine distinct neuroendophenotypes? *Biological Psychiatry* 2005; 57:143S-4S.
31.Pezawas L, Meyer-Lindenberg A, Drabant EM *et al*. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience* 2005; 8:828-34.
32.Hariri AR. Genetic susceptibility factors for intermediate phenotypes of serotonergic neurotransmission, amygdala reactivity, and fearful temperament. *Biological Psychiatry* 2004; 55:192S.
33.Holmes A, Hariri AR. The serotonin transporter gene-linked polymorphism and negative emotionality: placing single gene effects in the context of genetic background and environment. *Genes Brain and Behavior* 2003; 2:332-5.
34.Hariri AR, Weinberger DR. Functional neuroimaging of genetic variation in serotonergic neurotransmission. *Genes Brain and Behavior* 2003; 2:341-9.
35.Hariri AR, Goldberg TE, Mattay VS *et al*. Brain-derived neurotrophic factor val(66)met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *Journal of Neuroscience* 2003; 23:6690-4.
36.Hariri AR, Kolachana BS, Goldberg TE *et al*. BDNF val(66)met genetic variation and the response of the human hippocampus. *Biological Psychiatry* 2003; 53:113S.
37.Hariri AR, Weinberger DR. Imaging genomics. *British Medical Bulletin* 2003; 65:259-70.
38.Hariri AR, Mattay VS, Tessitore A *et al*. Serotonin transporter genetic variation and the response of the human amygdala. *Science* 2002; 297:400-3.
39.Hirvonen J, Van Erp TG, Huttunen J *et al*. Brain dopamine D1 receptors in twins discordant for schizophrenia. *American Journal of Psychiatry* 2006; 1631:1747-53.
40.de GE, Goldberg T, Boomsma DI, Posthuma D. Imaging the genetics of brain structure and function. *Biological Psychology* 2008; 79:1-8.
41.Hariri AR, Weinberger DR. Functional neuroimaging of genetic variation in serotonergic neurotransmission. *Genes Brain Behavior* 2003; 2:341-9.
42.Winterer G, Hariri AR, Goldman D, Weinberger DR. Neuroimaging and human genetics. *International Review of Neurobiology* 2005; 67:325-83.
43.Barnes J, Scahill RI, Frost C, Schott JM, Rossor MN, Fox NC. Increased hippocampal atrophy rates in AD over 6 months using serial MR imaging. *Neurobiology of Aging* 2008; 29:1199-203.

44.Toledo-Morrell L, Stoub TR, Wang CS. Hippocampal atrophy and disconnection in incipient and mild Alzheimer's disease. *Dentate Gyrus: A Comphrehensive Guide to Structure, Function, and Clinical Implications* 2007;163:741-+.
45.Tapiola T, Pennanen C, Tapiola M *et al*. MRI of hippocampus and entorhinal cortex in mild cognitive impairment: A follow-up study. *Neurobiology of Aging* 2008; 29:31-8.
46.Honea RA, Meyer-Lindenberg A, Hobbs KB *et al*. Is gray matter volume an intermediate phenotype for schizophrenia? A voxel-based morphometry study of patients with schizophrenia and their healthy siblings. *Biological Psychiatry* 2008; 63:465-74.
47.Konarski JZ, Mcintyre RS, Kennedy SH, Rafi-Tari S, Soczynska JK, Ketter TA. Volumetric neuroimaging investigations in mood disorders: bipolar disorder versus major depressive disorder. *Bipolar Disorders* 2008; 10:1-37.
48.Letizia B, Maricla T, Sara C *et al*. Magnetic resonance imaging volumes of the hippocampus in drug-naive patients with post-traumatic stress disorder without comorbidity conditions. *Journal of Psychiatric Research* 2008; 2:752-62.
49.Yamasue H, Abe O, Suga M *et al*. Gender-common and -specific neuroanatomical basis of human anxiety-related personality traits. *Cerebral Cortex* 2008; 8:46-52.
50.Jack CR, Bentley MD, Twomey CK, Zinsmeister AR. Mr Imaging-Based Volume Measurements of the Hippocampal-Formation and Anterior Temporal-Lobe - Validation Studies. *Radiology* 1990; 176:205-9.
51.Pantel J, O'Leary DS, Cretsinger K *et al*. A new method for the *in vivo* volumetric measurement of the human hippocampus with high neuroanatomical accuracy. *Hippocampus* 2000; 10:752-8.
52.Watson C, Andermann F, Gloor P *et al*. Anatomic Basis of Amygdaloid and Hippocampal Volume Measurement by Magnetic-Resonance-Imaging. *Neurology* 1992; 42:1743-50.
53.Ashburner J, Friston KJ. Voxel-based morphometry - The methods. *Neuroimage* 2000 June; 11:805-21.
54.Jezzard P, Matthews P M, Smith S M. *Functional MRI- An introduction to methods, New York: Oxford University Press,* 2001.
55.Logothetis NK, Wandell BA. Interpreting the BOLD signal. *Annual Review of Physiology* 2004; 66:735-69.
56.Logothetis NK. What we can do and what we cannot do with fMRI. *Nature* 2008; 453:869-78.

SECTION A

Functional Neuroimaging

2

Amygdala responses to emotional faces in twins discordant or concordant for the risk for anxiety and depression

Wolfensberger SP, Veltman DJ, Hoogendijk WJ,
Boomsma DI, de Geus EJ

Neuroimage. 2008 Jun;41(2):544-52. Epub 2008 Feb 14

Abstract

Background

Functional brain imaging studies have shown deviant amygdala responses to emotional stimuli in subjects suffering from anxiety and depressive disorder, but both hyperactivity and hypoactivity compared to healthy controls have been reported. To account for these discrepant findings, we hypothesize that genetic and environmental risk factors differently impact on amygdala functioning.

Methods

To test this hypothesis, we assessed amygdala responses to an emotional faces paradigm during functional magnetic resonance imaging in monozygotic twin pairs discordant for the risk of anxiety and depression (n =10 pairs) and in monozygotic twin pairs concordant for high (n=7 pairs) or low (n=15 pairs) risk for anxiety and depression.

Results

Main effects (all faces vs. baseline) revealed robust bilateral amygdala activity across groups. In discordant twins, increased amygdala responses were found for negatively valenced stimuli (angry/anxious faces) in high-risk twins compared to their low-risk co-twins. In contrast, concordant high-risk pairs revealed blunted amygdala reactivity to both positive and negative faces compared with concordant low-risk pairs. Post-hoc analyses showed that these findings were independent of 5-HTTLPR genotype.

Conclusions

Our findings indicate amygdala hyperactivity in subjects who are at high risk for anxiety and depression through environmental factors and amygdala hypoactivity in those at risk mainly through genetic factors. We suggest that this result reflects a difference in baseline amygdala activation in these groups. Future imaging studies on anxiety and depression should aim to avoid admixture of subjects who are at genetic risk with those at risk due to environmental factors.

Introduction

Functional imaging studies of brain correlates of anxiety and depression have converged on the amygdala, a structure crucial for emotional processing (Canli *et al.*, 2005; Davidson *et al.*, 2003; Drevets, 2001; Fu *et al.*, 2004; Kumari *et al.*, 2003; Lawrence *et al.*, 2004; Rauch *et al.*, 2000; Shin *et al.*, 2005; Siegle *et al.*, 2001; Stein *et al.*, 2002; Surguladze *et al.*, 2005; Thomas *et al.*, 2001; Wright *et al.*, 2003). A replicated association has been found between amygdala reactivity to emotional stimuli and a polymorphism in the serotonin transporter gene (Hariri *et al.*, 2002, 2005; Pezawas *et al.*, 2005), and other genes in the serotonergic pathway also seem to influence amygdala reactivity (Brown *et al.*, 2005; Buckholtz *et al.*, 2007; Dannlowski *et al.*, 2007; Iidaka *et al.*, 2005). tioning. About 60% of the risk for anxiety and depressive disorders can be attributed to environmental factors (Kendler and Prescott, 1999; Sullivan *et al.*, 2000) and interaction of genetic and environmental factors has also been reported (Caspi *et al.*, 2000; Eley *et al.*, 2004; Grabe *et al.*, 2005; Kaufman *et al.*, 2006; Kendler *et al.*, 1986).

It is currently unclear whether environmental risk factors have the same pathogenic effects as genetic risk factors. It is quite possible that these two types of risk impact on the same brain structures but in entirely different perhaps even opposite ways. Indeed, deviant amygdala responses to emotional stimuli in subjects suffering from anxiety and depressive disorder have been observed, but although most studies have found hyperactivity compared to healthy controls (Canli *et al.*, 2005; Fu *et al.*, 2004; Siegle *et al.*, 2001; Surguladze *et al.*, 2005), in others negative findings (Kumari *et al.*, 2003; Lawrence *et al.*, 2004; Davidson *et al.*, 2003), or even amygdala hypoactivity has been reported (Drevets, 2001; Thomas *et al.*, 2001). Such discrepant findings may well arise because genetic and environmental risk factors differently impact amygdala functioning.

Here we employ brain imaging of concordant and discordant monozygotic (MZ) twin pairs to test how genetic and environmental risks for anxiety and depression are reflected in the amygdala response to emotionally salient stimuli. Monozygotic twins are (nearly) always 100% identical at the DNA sequence level (Boomsma *et al.*, 2002) and differences in phenotypic status can be expected to reflect discordance for environmental risk factors. Discordant MZ twins were selected to be at low or high risk for anxiety disorder and major depression based on their ratings on neuroticism, anxiety, and depression in longitudinal surveys. A compound risk score for anxiety and depression based on these ratings was shown to have strong predictive validity for clinical anxiety and clinical depression in this population (Middeldorp *et al.*, 2004) as assessed by the Composite International Diagnostic Interview, a well-validated instrument to assess these disorders (Andrews and Peters, 1998). Because MZ twins are genetically identical, discordance in the risk for anxiety and

depression must arise from differential exposure to unique environmental influences. Our design allows us to test the hypothesis that these influences affect emotional processing by the amygdala.

In addition to discordant pairs, amygdala responses were assessed in two comparison groups of concordant monozygotic twin pairs. In the concordant high-risk group, both MZ twins scored high on neuroticism, anxiety, and depression in the longitudinal surveys. In the concordant low-risk group, both MZ twins had scored low on neuroticism, anxiety, and depression. The contrast between these two groups seems to mainly reflect a difference in genetic risk rather than in environmental risk because parents of the high-risk twins also scored very high on neuroticism, anxiety, and depression in the longitudinal surveys, whereas parents of the low-risk twins scored very low (De Geus *et al.*, 2006). Comparison of the low and high scoring subjects allowed us to extend the finding of differential amygdala function in patients vs. controls to extreme scoring subjects in the normal population. Furthermore, comparing the contrast in amygdalar response between concordant high- and low-risk pairs to the within-pair contrast in discordant pairs allowed us to test whether genetic and environmental risk factors have a similar effect on amygdala responses to emotional stimuli.

Material and methods

Subjects

Selection of subjects has been described in detail by De Geus *et al.* (2006). Briefly, in a sample of 2455 same sex twin pairs registered in the Netherlands Twin Registry, a compound risk score for anxiety and depression was computed based on a genetic factor analysis of longitudinal survey data on trait anxiety, depression, neuroticism, and somatic anxiety (for details, see Boomsma *et al.*, 2000). The surveys were collected in 1991, 1993, 1997, and 2000. An average risk score was computed for each twin across all available surveys (which could vary from one to four surveys).

Discordant MZ twins pairs were considered eligible for participation if both were right-handed, the high-risk subject had a risk score at least 0.5 standard deviation above the mean, and the score of high-and low-risk twins was at least 2 standard deviations apart. To estimate the relative risk for actual anxiety and depression disorder in twins ascertained by these criteria, we selected all subjects with comparable low-or high-risk scores from the larger sample of 1256 subjects that underwent a CIDI interview in 2000 (Middeldorp *et al.*, 2004). To resemble the observed mean risk scores in our discordant twins (-0.62 vs. 0.97, SD = 0.74), we selected subjects with a risk score of at least 1.5 standard deviation above the mean (1.06) on the 1991, 1993, and 1997 surveys and subjects with a risk score of at least 0.5 standard deviations below the mean (-0.32). In this sample, the relative risk to receive a lifetime depression diagnosis in the high scoring subjects was 11.8 compared with low scoring subjects and 40.1 for generalized anxiety disorder.

A total of 31 discordant MZ twin pairs met our criteria, of which 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 out of time constraints. One pair turned out to be dizygotic. This left a final 10 MZ pairs who were extremely discordant for the risk for anxiety and depression. Notably, the risk scores of the high-risk twin in these final 10 pairs did not differ from the risk scores in the high-risk twin from the original 31 selected pairs.

Concordant MZ twin pairs were considered eligible for participation if both were right-handed and both their risk scores were at least 0.8 standard deviation above (high risk) or below (low risk) the mean risk score. An even more stringent selection of extreme risk scores was possible here compared to the discordant MZ twin selection, because extreme scoring concordant MZ pairs are much less rare than extremely discordant pairs. This yielded 115 concordant high-risk and 137 concordant low-risk pairs, of which we invited 48 pairs that 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 refused to participate, mainly out of time constraints. This left a final 15 MZ pairs who were concordant for low risk and 7 MZ pairs who were concordant for high risk for anxiety and depression. Of the total group of 64 MZ, 28 were male and the mean age was 30 years (range 20–42 years).

Procedure

Subjects visited the outpatient MR unit and experimental procedures were explained in detail. Twins from the same pair always came on the same day. Twins were randomly assigned to an MRI scan session or a psychometric session. After about 90 min, twins switched between sessions. During the psychometric session, cognitive abilities and current psychiatric diagnostic state were assessed. All subjects were interviewed using the Composite International Diagnostic Interview (Peters and Andrews, 1995; Wittchen, 1994). The Montgomery Asberg Depression Rating Scale and the Beck Depression Inventory were used to assess depressive symptom characteristics and severity scores (Beck *et al.*, 1961; Montgomery and Asberg, 1979). Furthermore, the state version of the State-Trait Anxiety Inventory (Spielberger *et al.*, 1970) was administered pre-and post-scanning. Verbal comprehension (IQ) and working memory (forward and backward recall scores of digit scan) were assessed using subtests of the Wechsler Adult Intelligence Scale (Wechsler, 1997).

Table 1: 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, no.	14/16	4/6	4/6	6/8
Age, mean, years	30.9	30.6	30.6	26.1
BDI depression, mean (SD)¶,*	1.1 (1.5)	2.7 (2.5)	9.7 (10.6)	8.0 (6.0)
MADRAS, mean (SD)¶,*	0.43 (1.5)	2.1 (2.5)	5.0 (3.9)	5.1 (7.8)
STAI State Anxiety before Scan session, mean (SD)¶,*	27.7 (5.0)	31.4 (5.3)	37.0(7.3)	36.7(9.8)
STAI State Anxiety after Scan session, mean (SD)¶,#,*	25.1 (4.7)	28.3 (5.5)	37.1 (8.1)	33.8 (8.4)
Verbal IQ subscale, mean (SD)	13.1 (2.5)	14.5 (2.2)	14.6 (2.2)	12.9 (3.9)
Working Memory IQ subscale, mean (SD)	7.6 (1.3)	8.9 (2.4)	8.3 (3.2)	8.6 (1.6)

¶ Significant difference between low-risk and high-risk concordants.

Significant difference between high-risk subjects from concordant and discordant pairs.

* Significant intrapair difference in discordant twins.

Finally, social support was measured using the Duke–UNC questionnaire (Broadhead *et al.*, 1988) and subjects were asked to recall the occurrence of 21 major life events. These included individual (e.g. maltreatment, disease, financial problems, job strain, relational problems) events as well as network-related events (e.g. disease or loss of close kin). Subjects were asked to locate these events in four temporal categories, i.e. whether they occurred the last six months, between 6 and 12 months, between 1 and 5 years, or more than 5 years ago. For each event, they indicated the impact on their lives on a 10-point visual analogue scale ranging from 'no impact' to 'extreme impact'.

During each MRI session, which lasted 45 min, subjects performed two tasks using emotionally relevant stimuli (faces and words). At first, a verbal memory task took place, followed by an emotional faces task. Between the encoding and recognition phase of the memory task, a structural MR scan was performed (De Geus *et al.*, 2006). At the end of both sessions, subjects were debriefed and received sets of buccal swabs to collect mucosal cells for DNA extraction. DNA was used to confirm zygosity using 11 highly polymorphic markers and to type the l/s polymorphism in the promoter of the 5HT transporter gene. The 5-HTTLPR genotype was assessed in 26 MZ pairs. The ethical review board of the VU medical center approved the study and all participants provided written informed consent.

Task paradigm

We used an event-related emotional faces paradigm known to reliably activate the anterior medial temporal lobe including the amygdala (Wright *et al.*, 2003). All subjects viewed human face stimuli: angry, fearful, happy, and neutral human faces (Ekman and Friesen, 2006), as well as a scrambled face, which was used as a baseline condition. Each face stimulus condition consisted of 10 pictures, and each picture was presented three times. Stimuli were randomized once and then presented in the same fixed order to all subjects. Stimuli were displayed for 2500 ms with a variable interstimulus interval (400–600 ms), to increase experimental power and to decrease expectancy effects. To control for overflow effects, we displayed a baseline stimulus after each one or two face pictures.

Subjects were requested to make sex judgments during presentation of face stimuli to control for attention differences. Baseline stimuli (scrambled faces) were imbedded with two arrows in the center of the screen, and subjects were asked to indicate whether the arrows pointed to the left or to the right. To ensure that participants were familiar with the procedure, the task was explained outside the scanner before fMRI was performed. No feedback regarding the answers was provided during the task.

Table 2: Regions showing significantly increased brain activation across groups during fMRI scanning (faces vs. baseline)

Region (cortex)	MNI coordinates			Z-value	BA
	X	*Y*	*Z*		
DLPFC L	-48	15	27	3.59*	45
DLPFC R	51	12	27	4.57*	45
VLPFC L	-36	27	0	3.50*	46
VLPFC R	–	–	–	–	–
Anterior cingulate	-6	9	45	3.12*	24
Amygdala L	-21	-6	-18	4.86*	
Amygdala R	21	-6	-15	3.94*	
Thalamus R	-6	-9	-3	3.87*	
Temporo-occipital L	-39	-60	-18	6.55*	4
Temporo-occipital R	39	-57	-18	7.68*	4
Occipital	-33	-90	-12	5.51*	18/19
	36	-87	-9	5.92*	
	-6	-93	9	7.08*	17

* Significant FDR corrected at $p<0.05$.
Cluster size threshold, 5 voxels; BA, Brodmann area; DLPFC, dorsolateral prefrontal cortex; L, left; R, right; VLPFC, ventrolateral prefrontal cortex.

Table 3: Comparison of the amygdala effects (faces vs. baseline) in low-risk and high-risk twins

Condordant pairs

Concordant low-risk twins > concordant high-risk twins

Contrast	Region	MNI coordinates	Z-score
All faces vs. baseline	L amygdala	-18 -3 -15	2.59#
	R amygdala	27 -6 -24	3.15*
		21 0 -6	2.97*
Angry/anxious faces vs. baseline	R amygdala	27 -3 -24	3.39*
Happy faces vs. baseline	R amygdala	24 0 -24	2.91*

Discordant pairs

High risk twin > low risk co-twin

Contrast	Region	MNI coordinates	Z-score
All faces vs. baseline	L amygdala	-21 0 -21	2.67*
Angry/anxious faces vs. baseline	L amygdala	-30 -6 -21	2.71*

* Results reported significant at uncorrected $p < 0.005$, cluster size threshold 5 voxels.
# p=0.0054.

Image acquisition

Magnetic resonance imaging was performed on a 1.5-T Sonata MR system (Siemens, Erlangen, Germany) with a standard RF receiver head coil. Stimuli were generated by a Pentium PC and projected on a screen at the end of the scanner table, which was seen through a mirror mounted above the subject's head. Two magnet-compatible four-key response boxes were used to record subject's performance and reaction times (RTs). To reduce motion artefacts, the subject's head was immobilized using foam pads.

For functional MRI, an echo planar imaging sequence (TR = 3.04 s; TE = 45 ms; matrix: 64 x 64; field of view: 192 x 192 mm; flip angle = 90°) was used, creating transversal whole-brain acquisitions (35 slices, 3 x 3-mm in-plane resolution, 2.5-mm slice thickness, 0.5-mm interslice gap). In total, 214 EPI volumes per subjects were scanned. In addition, a coronal 3D gradient-echo T1-weighted sequence was performed (matrix: 256 x 256, inversion time: 300 ms, TR = 15 ms, TE = 7 ms; flip angle = 8°; voxel size, 1 x 1 x 1.5 mm, 160 slices).

Data analysis

Differences in the questionnaire and interview based variables and in the performance data (reaction times) during the faces task were examined by a mixed model ANOVA (MIXED SPSS) with type of twin pair (Discordant, Concordant low-risk, Concordant high-risk) and risk score level (High, Low) as two fixed factors and family as a random factor to account for within-family dependence. Primary planned contrasts were the comparison of the low-risk vs. the high-risk co-twin within the 10 discordant pairs, and of the 7 concordant high-risk vs. the 15 concordant low-risk pairs.

Imaging data were analyzed using SPM2 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK). After the first two volumes were discarded to allow for magnetic saturation, time series were corrected for differences in slice acquisition times and realigned. Next, data were warped to MNI space as defined by the SPM EPI template, and spatially smoothed using an 8 mm FWHM Gaussian kernel. After spatial preprocessing, data were analyzed using delta functions convolved with a canonical hemodynamic response function to model responses to each stimulus type. For each subject, weighted contrasts were computed for simple main effects. This was done across all stimulus types, i.e. viewing angry/anxious, happy, and neutral faces vs. baseline, and within stimulus type, e.g. angry/ anxious vs. baseline. The resulting contrasts images were entered into second level (random effects) analyses for between-group comparisons. These used a one-way ANOVA for the groups of concordant high-vs. low-risk pairs (Group) and paired *t*-tests for the high-risk twin vs. the low-risk co-twin within discordant pairs (Twin). SPM2's non-sphericity option was used to account for within-pair correlated observations. Main effects of risk status (Group or Twin) are reported at $p<0.05$ FDR-corrected for multiple comparisons with an extent threshold of five voxels (Genovese *et al.*, 2002). Interactive effects of Risk by stimulus type (angry/anxious vs. baseline, happy vs. baseline, neutral vs. baseline; masked with the relevant main effect) within the amygdala region are reported at $p<0.005$ uncorrected, also with an extent threshold of five voxels.

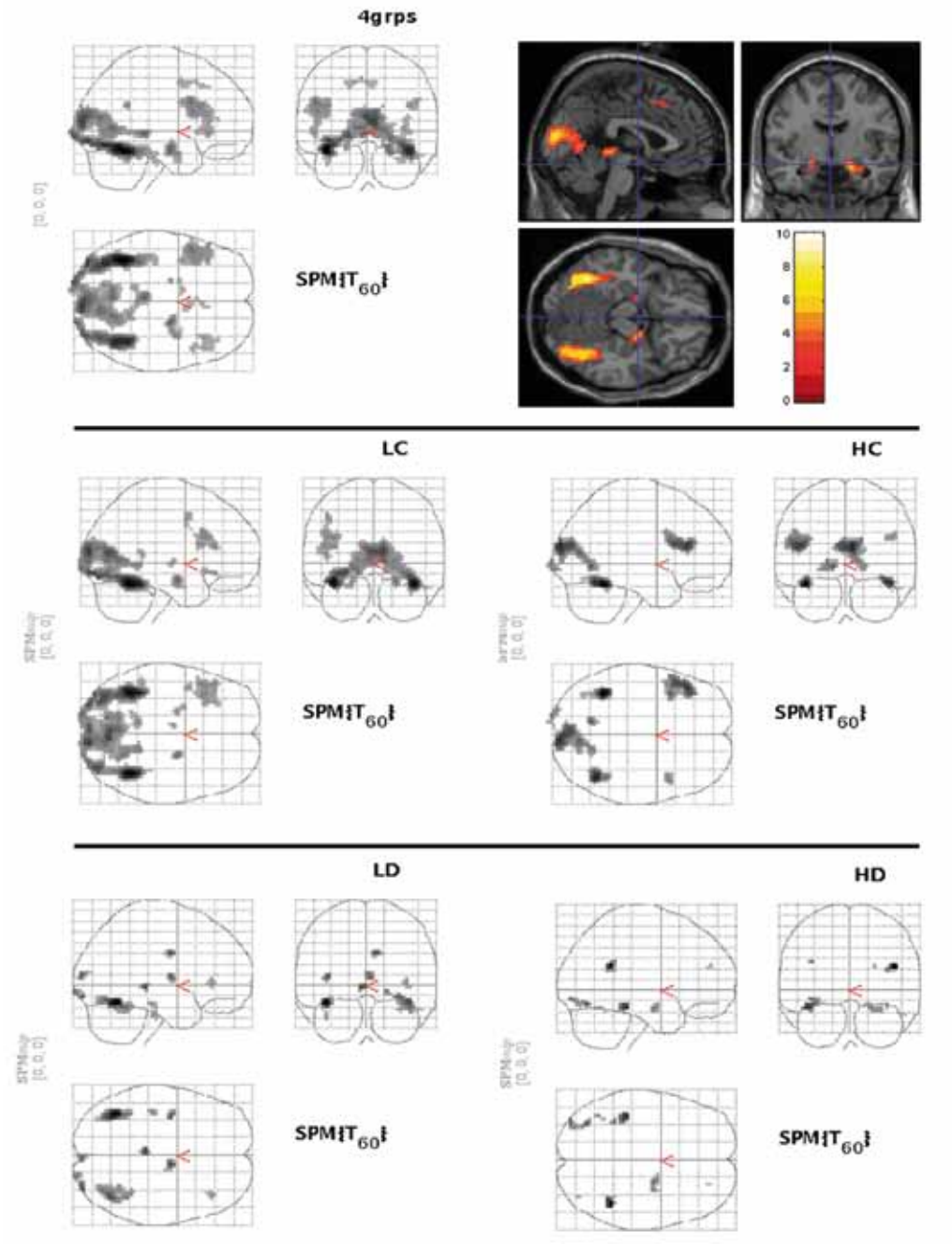

Figure 1: Main effect of faces (all faces vs. baseline) across all 4 experimental groups (upper left panel: glass brain; upper right panel: overlaid on T1-weighted anatomical image) and for the subgroups of the concordant low-risk pairs (LC, middle left panel), the concordant high-risk pairs (HC, middle right panel), the discordant low-risk twins (LD, lower left panel), and their high-risk co-twins (HD, lower right panel).

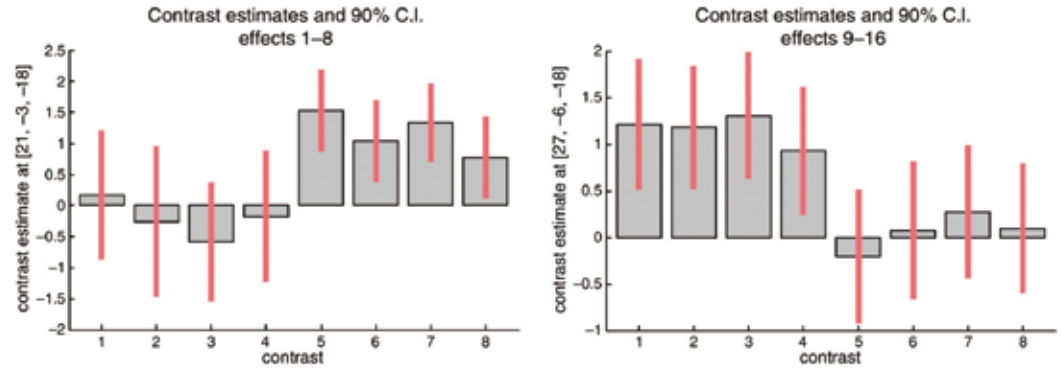

Figure 2: Left panel: parameter estimates (% signal change) and 90% confidence intervals for angry/anxious/happy/neutral faces vs. baseline (scrambled faces) inconcordant high-risk (effects 1–4) vs. concordant low-risk twins (effects 5–8). Right panel: parameter estimates (% signal change) and 90% confidence intervals for angry/anxious/happy/neutral faces vs. baseline (scrambled faces) in concordant high-risk (effects 9–12) vs. concordant low-risk twins (effects 13–16).

Results

Demographic data and depression/anxiety ratings (BDI, MADRS, STAI-state) obtained at the time of the scanning session are listed in Table 1. Age and male/female distribution was not significantly different across the groups. Only one subject in a concordant high-risk pair received a current diagnosis of depression using these instruments. One further subject currently used antidepressant medication (SSRI). This was the subject with the high-risk 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 the concordant high-risk pairs. Within the discordant pairs, the MADRS, BDI, and state anxiety measures all showed significant intra-pair differences in the expected direction.

Reaction times during the task (sex judgments) for angry, anxious, happy, neutral faces, and across categories did not differ significantly between groups (mean RT±SD: angry faces, 800±171 ms; anxious faces, 787±170 ms; happy faces, 794±172 ms; neutral faces, 769± 173 ms; across categories, 787±166 ms; baseline, 698±132 ms).

Imaging data

Effects of faces

Across groups, viewing faces compared to baseline were associated with robust activity in bilateral occipital cortex, extending into fusiform gyrus and posterior parahippocampal gyrus, as well as in bilateral amygdala, with additional activity in predominantly left lateral prefrontal cortex, dorsal anterior cingulate cortex, and thalamus (Table 2). These effects are depicted across groups (Fig. 1, upper panel) and for individual groups (Fig. 1, middle and lower panels).

Within-pair comparison of the discordant twins

Across the neutral, happy, and angry/anxious faces, paired *t*-tests comparing the low-risk twin to the high-risk co-twin showed increased left amygdala activity in the high-risk twin (Table 3). Risk x stimulus type interaction analyses in discordant twins revealed increased left amygdala activity for angry/anxious faces in high-risk twin compared to the low-risk co-twin, whereas this comparison failed to reach significance for happy or neutral faces (Fig. 2, left panel). High-risk twins had substantially higher state anxiety levels before the scan compared to their low-risk co-twin (see Table 1). Rerunning the analyses using these state anxiety levels as a covariate rendered all amygdala effects non-significant.

Group comparison of concordant high-risk and low-risk twin pairs

Across the neutral, happy, and angry/anxious faces, we observed increased activity over baseline in bilateral amygdala for concordant low-risk pairs compared to high-risk pairs (using one-way ANOVA), with left amygdala approaching significance (Table 3). Risk x stimulus type interaction analyses showed increased amygdala activity in low-risk pairs compared to the high-risk pairs for angry/anxious faces and happy faces relative to baseline, but not for neutral faces (Fig. 2, right panel). In contrast to the findings in discordant twins, rerunning the analyses using state anxiety levels before the scan as a covariate left all group differences in amygdala reactivity significant.

Table 4: 5-HTTLPR genotype in the three types of MZ twin pairs

	5-HTTLPR genotype			
	LL	LS/SL	SS	Total
Discordant twin pairs	4	12	2	18
Concordant high-risk pairs	4	4	0	8
Concordant low-risk pairs	8	14	4	26
Total pairs	16	30	6	52

Additional post hoc analyses showed highly similar results after excluding the subject meeting criteria for clinical depression and the subject using antidepressants. In addition, in a secondary analysis, we failed to observe amygdala activity when contrasting baseline trials with neutral faces in either the comparison of the low-risk twin with the high-risk co-twin in discordant pairs or in the comparison of concordant low-risk and high-risk pairs.

5-HTTLPR genotypes

Previous studies have reported differential amygdala responsivity in subjects with different genotypes for a functional polymorphism in the gene coding for the serotonin transporter (LL vs. LS vs. SS; Munafò *et al.*, 2007). Because the frequency of the LL genotype in the concordant high-risk pairs is high compared to that found in unselected samples, part of the attenuated amygdale response may have reflected effects of this particular polymorphism. To test whether our findings reflected effects of the 5-HTTLPR genotype, we first examined the 5-HTTLPR genotype distribution across groups (Table 4). Permutation tests showed that 5-HTTLPR genotype was not related to twin depression status (low or high risk for anxiety and depression). Next, we examined whether the amygdala response differed as a function of genotype by contrasting the BOLD responses of the LL (n = 8 pairs) and SL/ SS (n = 18 pairs) 5-HTTLPR genotypes. This was done independent of risk or concordance status, i.e. pooled across the four groups. No significant differences between genotype groups were found.

Discussion

The main aim of this study was to investigate differences in amygdala response to emotional faces in relation to a “pure” environmental risk for depression and anxiety and to contrast this with the amygdala response in relation to genetic risk. All subjects viewed positively and negatively valenced emotional and neutral faces during functional MR imaging. Analysis of imaging data revealed robust activation in visual processing areas including the fusiform face area as well as in bilateral amygdala, left ventrolateral prefrontal cortex, and bilateral dorsolateral prefrontal cortex, the latter presumably reflecting sex categorization (Adams and Janata, 2002). These results are in agreement with a number of previous studies using a similar paradigm. Specifically, although in some studies predominantly right-sided responses were observed, irrespective of emotion (Gur *et al.*, 2002), in others bilateral amygdala responses were found across stimulus conditions (Britton *et al.*, 2006; Fitzgerald *et al.*, 2006; Yang *et al.*, 2002), similar to the present study.

In discordant MZ twin pairs, increased left amygdala responsiveness was found in the high-risk twin for angry/anxious faces. As MZ twins are nearly always genetically identical, this

amygdala hyperactivity in the high-risk twin necessarily reflects the role of environmental factors. Indeed, as reported previously (De Geus *et al.*, 2006), nine out of the ten high-risk twins from the discordant pairs reported major life events that occurred more than 5 years ago, and 5 twins reported multiple events. From the low-risk co-twins, only four reported such events, and only one reported multiple events. In addition, the average impact of the life events was generally rated to be larger by the high-risk twins. In keeping with these findings, amygdala hyperactivity in response to faces has been found in posttraumatic stress disorder (Rauch *et al.*, 2000; Siegle *et al.*, 2001), in which life events are by definition of aetiological significance, but also in social phobia and generalized anxiety disorder (Stein *et al.*, 2002), in which the role of genetic factors may be only modest (Middeldorp *et al.*, 2004; Mackintosh *et al.*, 2006). In contrast, normal or even lowered amygdala responses to emotional faces have been reported in simple phobias (Wright *et al.*, 2003) and obsessive–compulsive disorder (Cannistraro *et al.*, 2004).

At odds with our findings in discordant twins, the concordant high-risk twin pairs had a blunted amygdala response to emotional faces. Assessment of parental data for all pairs confirmed higher values for all risk traits in the parents of the concordant high-risk twins compared to the parents of the concordant low-risk twins (De Geus *et al.*, 2006). Although additional exposure of the high-risk twin pairs cannot be excluded, this strongly suggests that the concordant pairs mainly represent a contrast in genetic risk. Hence, we observed a clear dissociation in the amygdala responses of subjects who were at high-risk for anxiety and depression through environmental causes (hyperactivity) compared to those mostly at risk through genetic factors (hypoactivity).

These clear differences in the effects of genetic and environmental risk factors might help explain the inconsistency in the outcome of previous fMRI studies using similar emotional paradigms in MDD patients and controls. Increased responses to negative emotional stimuli in depressed subjects were observed by several investigators (Canli *et al.*, 2005; Fu *et al.*, 2004; Siegle *et al.*, 2001; Surguladze *et al.*, 2005), although decreased responding to positive emotional stimuli (happy faces) has also been found, which was associated with anhedonia severity (Surguladze *et al.*, 2005). Other groups, however, failed to observe differential amygdala activity (Davidson *et al.*, 2003; Kumari *et al.*, 2003; Lawrence *et al.*, 2004) or even reported a blunted response, in depressed children (Thomas *et al.*, 2001) and familial depression (Drevets, 2001), respectively. These inconsistencies have been attributed to methodological issues (e.g. medication use), or differences in state anxiety (Sheline *et al.*, 2001). In the present study, medication use was virtually absent in the high-risk twins which rules this out as an explanation here. Anxiety ratings were high in both discordant high-risk twins and concordant high-risk twin pairs which suggest that state anxiety cannot fully account for the contrast between hyporeactivity and hyperreactivity. Instead, genetic and environmental risk factors, although converging on the same pathway, seem to exert different effects on amygdala functioning. Hence, differential exposure of patients to genetic or environmental risk factors, perhaps related to ascertainment methods, may account for part of the differential findings in the literature.

Previous research in genetically contrasted groups has revealed larger amygdala activity in response to emotional faces in subjects with one or two copies of the short allele of the serotonin transporter promoter (HTTLPR) polymorphism (Bertolino *et al.*, 2005; Hariri *et al.*, 2002, 2005). Since the short allele carriers have an increased risk for anxiety and depression compared to long allele homozygotes, particularly in interaction with life stress (Caspi *et al.*, 2003), it might be argued that our findings could be explained by differential genotypes for the serotonin transporter gene in the three groups of twins (concordant low-risk, concordant high-risk, discordant). This was not the case, as there was no difference in the distribution of the HTTLPR alleles between the three groups. This is not without precedent since amygdala responsiveness has previously been found to be modulated by personality style (Bertolino *et al.*, 2005) and harm avoidance (Hariri *et al.*, 2005), independent of the 5-HTTLPR genotype. Also, a direct test of 5-HTTLPR genotype effects on the amygdala response was nonsignificant. This may reflect the small sample size, but null findings have been noted before (Munafò *et al.*, 2007).

This leaves us to explain the attenuated amygdala responses in the concordant high-risk twins, a group selected to be at high genetic risk for anxiety and depression. Based on the proposed inverse relationship between amygdala reactivity and baseline amygdala activation (Canli *et al.*, 2006), we hypothesize that this genetic risk co-occurs with an increase in baseline amygdala activation. This is in keeping not only with recent perfusion MRfindings (Canli *et al.*, 2006) showing increased baseline amygdala perfusion in subjects with decreased responsiveness to emotional stimuli, but also with SPECT-and PET-resting-state studies in familial MDD patients by Drevets and coworkers (Drevets, 2000a,b, 2001, 2003). A consistent finding in these latter studies has been elevated baseline amygdala perfusion and/or glucose uptake in patients with familial MDD (Drevets, 2000 a,b, for an overview). The mechanism of increased amygdala baseline metabolism in familial MDD is insufficiently understood, but may reflect both altered afferent transmission (increased glutamatergic input from ventral prefrontal areas and/or decreased inhibitory control from dorsal medial PFC) as well as a shift from ISPS to ESPS within the amygdala due to elevated CRF secretion (Drevets, 2003; Phillips *et al.*, 2003; Shekhar *et al.*, 2003). Animal research has indicated that the increase in regional CBF or metabolism may be as high as 50–70% (Drevets, 2003). Although this is still in the physiological range, it is conceivable that a rise in baseline perfusion of such magnitude may compromise amygdala responsiveness during processing of emotional stimuli. At present, such an explanation is speculative, since studies

in MDD assessing both amygdala resting state perfusion and responsiveness to emotional stimuli have been rare (Anand *et al*., 2005). Moreover, it should be taken into account that only one of our concordant high-risk twins met criteria for major depression at the time of scanning, so that extension of our findings of blunted amygdala responsiveness to clinical studies should be done with caution.

Various limitations of this study should be discussed. First, it is important to note that, although our selection of high-and low-risk twins was based on trait measures, the high-and low-risk twins were also strongly discriminated in state anxiety levels at the time of testing. This is an expected outcome. Individuals high on trait scores for neuroticism, anxiety, and depression are expected to respond to the study setting (unfamiliar environment, imperfect prediction of things to come, unknown experimenter, intimidating MRI apparatus, etc.) with more anxiety then individuals with low scores for these traits. It is of note that these state anxiety differences do not escape the basic tenet of our design: they will derive from differential environmental exposure in the discordant pairs and mainly derive from a difference in genetic make-up in the concordant pairs. Nonetheless, the effects of risk status defined by the longitudinal assessed trait values were obviously confounded with the effects of state anxiety. In an attempt to separate the effects of trait from state effects, we reran the analyses using state anxiety before the scan as a covariate. This was expected to largely undo the effects of our careful longitudinal selection for high trait values in both genetic and environmental contrasts. Instead, hypoactivity in the high-risk concordant twins remained significant, whereas hyperactivity of the high-risk discordant twin disappeared. This suggests that high state anxiety is responsible for amygdala hyperactivity in subjects at risk through environmental factors. They behave as healthy subjects deliberately made anxious by employing aversive conditioning paradigms. Increased amygdala responsiveness has consistently been observed in such paradigms (Buchel *et al*., 1998, 1999; Schienle *et al*., 2005). In contrast, the increased baseline activation in subjects at genetic risk seems to completely overrule these normal effects of state anxiety. Only a design that manages to assess the effects of state and trait anxiety independently can fully resolve this issue.

As a second limitation, it should be noted that blunted amygdala responses in high-concordant subjects could be due to increased amygdala responsiveness to the baseline trials, as was recently demonstrated by Heinz *et al*. (2007). In their study, these authors showed that in s-allele carriers, amygdala activation was increased during passively viewing a fixation cross relative to neutral stimuli, explained as resulting from experiencing (stressful) uncertainty. However, in the present study, post-hoc analyses failed to show amygdala activity when contrasting baseline trials with neutral faces. We avoided the use of unconstrained baseline trials (Stark and Squire, 2001) by requesting subjects to report the direction of arrows (overlaid on scrambled faces) which apparently attenuates the effect of

the uncertainty generated by fixation cross type baseline stimuli.

Finally, we acknowledge that the discordant MZ twins may not have been perfect genocopies. A number of rare occurrences can lead to genetic differences between MZ twins (Martin *et al*., 1997)and such MZ twin pairs may be enriched in a sample that is highly selected for trait discordance. If these MZ twins were not genetically identical this would question the role of the environment in making them discordant. In contrast to this idea and in keeping with true differences in environmental exposure, we found that the high-risk discordant twins had been exposed to adverse life events more often than their low-risk co-twin. Also, the most likely source of genetic differences within twin-pairs, epigenetics, seems to be primarily driven by differential environmental exposure itself (Fraga *et al*., 2005).

In summary, we observed decreased amygdala reactivity to emotional (positive and negative) faces in twins at high genetic risk for anxiety and depression, whereas increased responsiveness to negatively valenced faces was observed in twins at high environmental risk. We suggest that this result reflects a difference in baseline amygdala activation in these two groups. Further research should aim to elucidate pathophysiological mechanisms of amygdala dysfunction in genetically vs. environmentally driven anxiety and depression. In addition, future imaging studies on anxiety and depression should avoid admixture of subjects who are at risk due to genetic factors with those at risk due to environmental factors.

References

1. Adams, R.B., Janata, P., 2002. A comparison of neural circuits underlying auditory and visual object categorization. NeuroImage 16, 361–377.
2. Anand, A., Li, Y., Wang, Y., Wu, J.W., Gao, S.J., Bukhari, L., Mathews, V.P., Kalnin, A., Lowe, M.J., 2005. Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. Biol. Psychiatry 57, 1079–1088.
3. Andrews, G., Peters, L., 1998. The psychometric properties of the Composite International Diagnostic Interview. Soc. Psychiatry Psychiatr. Epidemiol. 33, 80–88.
4. Beck, A.T., Erbaugh, J., Ward, C.H., Mock, J., Mendelsohn, M., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561–571.
5. Bertolino, A., Arciero, G., Rubino, V., Latorre, V., De Candia, M., Mazzola, V., Blasi, G., Caforio, G., Hariri, A., Kolachana, B., Nardini, M., Weinberger, D.R., Scarabino, T., 2005. Variation of human amygdala response during threatening stimuli as a function of 5'HTTLPR genotype and personality style. Biol. Psychiatry 57, 1517–1525.
6. Boomsma, D.I., Beem, A.L., van den, B.M., Dolan, C.V., Koopmans, J.R., Vink, J.M., de Geus, E.J., Slagboom, P.E., 2000. Netherlands twin family study of anxious depression NETSAD. Twin Res. 3, 323–334.
7. Boomsma, D., Busjahn, A., Peltonen, L., 2002. Classical twin studies and beyond. Nat. Rev., Genet. 3, 872–882.
8. Britton, J.C., Taylor, S.F., Sudheimer, K.D., Liberzon, I., 2006. Facial expressions and complex IAPS pictures: common and differential networks. NeuroImage 31, 906–919.
9. Broadhead, W.E., Gehlbach, S.H., Degruy, F.V., Kaplan, B.H., 1988. The Duke-Unc Functional Social Support Questionnaire—measurement of social support in family medicine patients. Med. Care 26, 709–723.
10. Brown, S.M., Peet, E., Manuck, S.B., Williamson, D.E., Dahl, R.E., Ferrell, R.E., Hariri, A.R., 2005. A regulatory variant of the human tryptophan hydroxylase-2 gene biases amygdala reactivity. Mol. Psychiatry 10, 884–888.
11. Buchel, C., Dolan, R.J., Armony, J.L., Friston, K.J., 1999. Amygdalahippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. J. Neurosci. 19, 10869–10876.
12. Buchel, C., Morris, J., Dolan, R.J., Friston, K.J., 1998. Brain systems mediating aversive conditioning: an event-related fMRI study. Neuron 20, 947–957.
13. Buckholtz, J.W., Callicott, J.H., Kolachana, B., Hariri, A.R., Goldberg, T.E., Genderson, M., Egan, M.F., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2007. Genetic variation in MAOA modulates ventromedial prefrontal circuitry mediating individual differences in human personality. Mol. Psychiatry 10, 893–895.
14. Canli, T., Cooney, R.E., Goldin, P., Shah, M., Sivers, H., Thomason, M.E., Whitfield-Gabrieli, S., Gabrieli, J.D.E., Gotlib, I.H., 2005. Amygdala reactivity to emotional faces predicts improvement in major depression. NeuroReport 16, 1267–1270.
15. Canli, T., Qiu, M., Omura, K., Congdon, E., Haas, B.W., Amin, Z., Herrmann, M.J., Constable, R.T., Lesch, K.P., 2006. Neural correlates of epigenesis. Proc. Natl. Acad. Sci. U. S. A. 103, 16033–16038.
16. Cannistraro, P.A., Wright, C.I., Wedig, M.M., Martis, B., Shin, L.M., Wilhelm, S., Rauch, S.L., 2004. Amygdala responses to human faces in obsessive–compulsive disorder. Biol. Psychiatry 56, 916–920.
17. Caspi, A., Taylor, A., Moffitt, T.E., Plomin, R., 2000. Neighborhood deprivation affects childrens mental health: Environmental risks identified in a genetic design. Psychol. Sci. 11, 338–342.
18. Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389.
19. Dannlowski, U., Ohrmann, P., Bauer, J., Deckert, J., Hohoff, C., Kugel, H., Arolt, V., Heindel, W., Kersting, A., Baune, B.T., Suslow, T., 2007. 5-HTTLPR biases amygdala activity in response to masked facial expressions in major depression. Neuropsychopharmacology 33, 418–424.
20. Davidson, R.J., Irwin, W., Anderle, M.J., Kalin, N.H., 2003. The neural substrates of affectiveprocessing in depressed patients treated with venlafaxine. Am. J. Psychiatry 160, 64–75.
21. De Geus, E.J., Ent, D.V., Wolfensberger, S.P., Heutink, P., Hoogendijk, W.J., Boomsma, D.I., Veltman, D.J., 2006. Intrapair differences in hippocampal volume in monozygotic twins discordant for the risk for anxiety and depression. Biol. Psychiatry 61, 1062–1071.
22. Drevets, W.C., 2000a. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. Prog. Brain Res. 126, 413–431.
23. Drevets, W.C., 2000b. Neuroimaging studies of mood disorders. Biol. Psychiatry 48, 813–829.
24. Drevets, W.C., 2001. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Curr. Opin. Neurobiol. 11, 240–249.
25. Drevets, W.C., 2003. Neuroimaging abnormalities in the amygdala in mood disorders. Ann. N.Y Acad. Sci. 985, 420–444.
26. Ekman, P., Friesen, W., 2006. Pictures of Facial Affect. Consulting Psychologists Press, Palo Alto.

27.Eley, T.C., Sugden, K., Corsico, A., Gregory, A.M., Sham, P., McGuffin, P., Plomin, R., Craig, I.W., 2004. Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol. Psychiatry 9, 908–915.

28.Fitzgerald, D.A., Angstadt, M., Jelsone, L.M., Nathan, P.J., Phan, K.L., 2006. Beyond threat: amygdala reactivity across multiple expressions of facial affect. NeuroImage 30, 1441–1448.

29.Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestart, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.Z., Plass, C., Esteller, M., 2005. Epigenetic differences arise during the lifetime of monozygotic twins. Proc. Natl. Acad. Sci. U. S. A. 102, 10604–10609.

30.Fu, C.H.Y., Williams, S.C.R., Cleare, A.J., Brammer, M.J., Walsh, N.D., Kim, J., Andrew, C.M., Pich, E.M., Williams, P.M., Reed, L.J., Mitterschiffthaler, M.T., Suckling, J., Bullmore, E.T., 2004. Attenuation of the neural response to sad faces in major depression by antidepressant treatment—a prospective, event-related functional magnetic resonance imaging study. Arch. Gen. Psychiatry 61, 877–889.

31.Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. NeuroImage 15, 870–878.

32.Grabe, H.J., Lange, M., Wolff, B., Volzke, H., Lucht, M., Freyberger, H.J., John, U., Cascorbi, I., 2005. Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden. Mol. Psychiatry 10, 220–224.

33.Gur, R.C., Schroeder, L., Turner, T., McGrath, C., Chan, R.M., Turetsky, B.I., Alsop, D., Maldjian, J., Gur, R.E., 2002. Brain activation during facial emotion processing. NeuroImage 16, 651–662.

34.Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., Weinberger, D.R., 2002. Serotonin transporter genetic variation and the response of the human amygdala. Science 297, 400–403.

35.Hariri, A.R., Drabant, E.M., Munoz, K.E., Kolachana, L.S., Mattay, V.S., Egan, M.F., Weinberger, D.R., 2005. A susceptibility gene for affective disorders and the response of the human amygdala. Arch. Gen. Psychiatry 62, 146–152.

36.Heinz, A., Smolka, M.N., Braus, D.F., Wrase, J., Beck, A., Flor, H., Mann, K., Schumann, G., Buchel, C., Hariri, A.R., Weinberger, D.R., 2007. Serotonin transporter genotype 5-HTTLPR: Effects of neutral and undefined conditions on amygdala activation. Biol. Psychiatry 61, 1011–1014.

37.Iidaka, T., Ozaki, N., Matsumoto, A., Nogawa, J., Kinoshita, Y., Suzuki, T., Iwata, N., Yamamoto, Y., Okada, T., Sadato, N., 2005. A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala. J. Neurosci. 25, 6460–6466.

38.Kaufman, J., Yang, B.Z., Douglas-Palumberi, H., Grasso, D., Lipschitz, D., Houshyar, S., Krystal, J.H., Gelernter, J., 2006. Brain-derived neurotrophic factor–5-HTTLPR gene interactions and environmental modifiers of depression in children. Biol. Psychiatry 59, 673–680.

39.Kendler, K.S., Prescott, C.A., 1999. A population-based twin study of lifetime major depression in men and women. Arch. Gen. Psychiatry 56, 39–44.

40.Kendler, K.S., Heath, A., Martin, N.G., Eaves, L.J., 1986. Symptoms of anxiety and depression in a volunteer twin population—the etiologic role of genetic and environmental-factors. Arch. Gen. Psychiatry 43, 213–221.

41.Kumari, V., Mitterschiffthaler, M.T., Teasdale, J.D., Malhi, G.S., Brown, R.G., Giampietro, V., Brammer, M.J., Poon, L., Simmons, A., Williams, S.C.R., Checkley, S.A., Sharma, T., 2003. Neural abnormalities during cognitive generation of affect in treatment-resistant depression. Biol. Psychiatry 54, 777–791.

42.Lawrence, N.S., Williams, A.M., Surguladze, S., Giampietro, V., Brammer, M.J., Andrew, C., Frangou, S., Ecker, C., Phillips, M.L., 2004. Subcortical and ventral prefrontal cortical neural responses to facial expressions distinguish patients with bipolar disorder and major depression. Biol. Psychiatry 55, 578–587.

43.Mackintosh, M.A., Gatz, M., Wetherell, J.L., Pedersen, N.L., 2006. A twin study of lifetime Generalized Anxiety Disorder (GAD) in older adults: genetic and environmental influences shared by neuroticism and GAD. Twin Res. Hum. Genet. 9, 30–37.

44.Martin, N., Boomsma, D., Machin, G., 1997. A twin-pronged attack on complex traits. Nat. Genet. 17, 387–392.

45.Middeldorp, C.M., Cath, D.C., Beem, A.L., Boomsma, D.I., 2004. Genetic epidemiology of depression in a selected population of Dutch twins and their siblings. Behav. Genet. 34, 652–653.

46.Montgomery, S.A., Asberg, M., 1979. New depression scale designed to be sensitive to change. Br. J. Psychiatry 134, 382–389.

47.Munafò, M.R., Brown, S.M., Hariri, A.R., 2007. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. Biol. Psychiatry (Oct 17, Electronic publication ahead of print).

48.Peters, L., Andrews, G., 1995. Procedural validity of the computerized version of the Composite International Diagnostic Interview Cidi-Auto in the Anxiety Disorders. Psychol. Med. 25, 1269–1280.

49.Pezawas, L., Angst, J., Kasper, S., 2005. Recurrent brief depression revisited. Int. Rev. Psychiatry 17, 63–70.
50.Phillips, M.L., Drevets, W.C., Rauch, S.L., Lane, R., 2003. Neurobiology of emotion perception: II. Implications for major psychiatric disorders. Biol. Psychiatry 54, 515–528.
51.Rauch, S.L., Whalen, P.J., Shin, L.M., McInerney, S.C., Macklin, M.L., Lasko, N.B., Orr, S.P., Pitman, R.K., 2000. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol. Psychiatry 47, 769–776.
52.Schienle, A., Schafer, A., Stark, R., Walter, B., Vaitl, D., 2005. Gender differences in the processing of disgust-and fear-inducing pictures: an fMRI study. NeuroReport 16, 277–280.
53.Shekhar, A., Sajdyk, T.J., Gehlert, D.R., Rainnie, D.G., 2003. The amygdala, panic disorder, and cardiovascular responses. Amygdala in Brain Function: Basic and Clinical Approaches, vol. 985, pp. 308–325.
54.Sheline, Y.I., Barch, D.M., Donnelly, J.M., Ollinger, J.M., Snyder, A.Z., Mintun, M.A., 2001. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. Biol. Psychiatry 50, 651–658.
55.Shin, L.M., Wright, C.I., Cannistraro, P.A., Wedig, M.M., McMullin, K., Martis, B., Macklin, M.L., Lasko, N.B., Cavanagh, S.R., Krangel, T.S., Orr, S.P., Pitman, R.K., Whalen, P.J., Rauch, S.L., 2005. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch. Gen. Psychiatry 62, 273–281.
56.Siegle, G.J., Granholm, E., Ingram, R.E., Matt, G.E., 2001. Pupillary and reaction time measures of sustained processing of negative information in depression. Biol. Psychiatry 49, 624–636.
57.Spielberger, C.D., Gorsuch, R.L., Lushene, R.E., 1970. STAI Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA.
58.Stark, C.E.L., Squire, L.R., 2001. When zero is not zero: the problem of ambiguous baseline conditions in fMRI. Proc. Natl. Acad. Sci. U. S. A. 98, 12760–12765.
59.Stein, M.B., Goldin, P.R., Sareen, J., Zorrilla, L.T.E., Brown, G.G., 2002. Increased amygdala activation to angry and contemptuous faces in generalized social phobia. Arch. Gen. Psychiatry 59, 1027–1034.
60.Sullivan, P.F., Neale, M.C., Kendler, K.S., 2000. Genetic epidemiology of major depression: review and meta-analysis. Am. J. Psychiatry 157, 1552–1562.
61.Surguladze, S., Brammer, M.J., Keedwell, P., Giampietro, V., Young, A.W., Travis, M.J., Williams, S.C.R., Phillips, M.L., 2005. A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. Biol. Psychiatry 57, 201–209.

62.Thomas, K.M., Drevets, W.C., Dahl, R.E., Ryan, N.D., Birmaher, B., Eccard, C.H., Axelson, D., Whalen, P.J., Casey, B.J., 2001. Amygdala response to fearful faces in anxious and depressed children. Arch. Gen. Psychiatry 58, 1057–1063.
63.Wechsler, D., 1997. WAIS-III Wechsler Adult Intelligence Scale. Psychological Corporation, San Antonio, Texas.
64.Wittchen, H.U., 1994. Reliability and validity studies of the Who Composite International Diagnostic Interview Cidi—a critical-review. J. Psychiat. Res. 28, 57–84.
65.Wright, C.I., Martis, B., McMullin, K., Shin, L.M., Rauch, S.L., 2003. Amygdala and insular responses to emotionally valenced human faces in small animal specific phobia. Biol. Psychiatry 54, 1067–1076.
66.Yang, T.T., Menon, V., Eliez, S., Blasey, C., White, C.D., Reid, A.J., Gotlib, I.H., Reiss, A.L., 2002. Amygdalar activation associated with positive and negative facial expressions. NeuroReport 13, 1737–1741.

3

The neural correlates of verbal encoding and retrieval in monozygotic twins at low or high risk for depression and anxiety

Wolfensberger SP, Veltman DJ, Hoogendijk WJ,
De Ruiter MB, Boomsma DI, de Geus EJ.

Biological Psychology. 2008 Sep;79(1):80-90. Epub 2008 Jan 18

Abstract

Emotional processing and brain activation were examined during an encoding and recognition paradigm using emotionally salient words in a sample of monozygotic twin pairs at low or high risk for anxiety and depression. Discordant twin pairs were used to chart the effects of environmental risk factors and concordant twin pairs were used to chart the effects of genetic risk factors on performance and brain activation.

Performance data did not support the existence of a negative response bias in subjects at high risk. At the neural level, however, increased left inferior frontal gyrus (LIFG) activation by negative words was found in high-risk subjects, most prominently during recognition. Increased LIFG activity was found in subjects at high risk through either genetic or environmental risk factors. These results suggest that fMRI activation of the LIFG in a verbal emotional memory task may be a useful vulnerability marker for anxiety and depression.

Introduction

Psychological theories of major depression have emphasized the role of negative biases in information processing in the etiology and the maintenance of the disorder (Beck, 1967). Such biases have been reported both for the interpretation and storage of emotional information (Bradley *et al.*, 1995; Leppanen, 2006; Murphy *et al.*, 1999; Phillips *et al.*, 2003). For example, in facial expression recognition tasks, depressed patients show a bias away from positive towards negative emotions, i.e. reduced recognition of positive expressions and increased perception of negative expressions (Gur *et al.*, 1992; Bouhuys *et al.*, 1995; Surguladze *et al.*, 2004). Negative perceptual and memory biases have also been found in healthy volunteers following negative mood induction (Bouhuys *et al.*, 1995; Teasdale and Russell, 1983). Furthermore, there is evidence that abnormal emotional processing may persist in the non-depressed state (Bhagwagar *et al.*, 2004; Hayward *et al.*, 2005; Leppanen, 2006) and is associated with poor outcome (Beevers *et al.*, 2007). These observations raise the possibility that emotional biases might pre-date the onset of clinical depression and thereby represent a risk factor for the subsequent development of illness in predisposed individuals.

To investigate emotional bias prior to the onset of depression it is necessary to study people who are at risk for anxiety and depression but who are not clinically depressed. Until now, studies combining brain imaging and neuropsychological testing in groups at high risk for depression and anxiety, but not yet affected, have been scarce. Moreover, these studies have focused primarily on executive functioning, whereas functional imaging studies investigating emotion processing in a memory paradigm have been lacking. Here we examined emotion processing, memory performance, and brain activation during an encoding and recognition paradigm using emotionally salient words in a sample of longitudinally followed monozygotic (MZ) twin pairs selected to be at low or high risk for anxiety disorder and major depression based on their ratings on neuroticism, anxiety, and depression in longitudinal surveys. A compound risk score for anxiety and depression based on these ratings was shown to have strong predictive validity for clinical anxiety and clinical depression in this population (Middeldorp *et al.*, 2004) as assessed by the Composite International Diagnostic Interview, a well-validated instrument to assess these disorders (Andrews and Peters, 1998). High-risk twins were included only, however, when they were not clinically depressed or anxious at the time of MRI recording, as assessed by a structured psychiatric interview.

About 60% of the risk for anxiety and depressive disorders can be attributed to environmental factors (Kendler and Prescott, 1999; Sullivan *et al.*, 2000) and interaction of genetic and environmental factors has also been reported (Caspi *et al.*, 2000; Eley *et al.*,

2004; Grabe *et al*., 2005; Kaufman *et al*., 2006; Kendler *et al*., 1986). It is currently unclear whether environmental risk factors have the same pathogenic effects as genetic risk factors. It is quite possible that these two types of risk impact on emotional processing but through entirely different routes. To separate the effects of genetic and environmental risk factors on emotional biases, we here employed the concordant and discordant twin pair design described earlier (de Geus *et al*., 2007). To detect the effects of environmental risk factors, performance and brain activation were compared in MZ twin pairs that were discordant for the risk for anxiety and depression. In these pairs, one twin scored high on neuroticism, anxiety, and depression in longitudinal surveys, whereas the co-twin scored low on these measures. Because monozygotic twins are (nearly) always 100% identical at the DNA sequence level (Boomsma *et al*., 2002), this discordance in the risk for anxiety and depression must arise from differential exposure to unique environmental influences. Differences in performance and brain activation during encoding and retrieval of emotionally salient words between the high-risk twin and the low-risk co-twin, therefore, must reflect environmental effects on emotional biases. To detect the effects of genetic risk factors on emotional biases we compared the performance and brain activation during encoding and recognition in twin pairs concordant for low risk with that in twin pairs concordant for high risk, i.e., both MZ twins of the pair scored low or high on neuroticism, anxiety, and depression in longitudinal surveys. The contrast between these two groups mainly reflects a difference in genetic risk rather than in environmental risk because parents of the high-risk twins also scored very high on neuroticism, anxiety, and depression in the longitudinal surveys, whereas parents of the low-risk twins scored very low (de Geus *et al*., 2007).

Neuroimaging studies of verbal encoding and recognition to date have found that the main neural correlates of semantic processing and retrieval of words are the left inferior frontal gyrus in healthy volunteers (LIFG, Fletcher and Henson, 2001), reflecting semantic elaboration in working memory, and the medial temporal lobe (MTL), including the fusiform gyrus and hippocampus (Squire and Knowlton, 2000), the latter thought to reflect intermediate memory storage. Taken the hypothesized emotional bias, we expected twins at high risk for anxiety and depression to show higher levels of semantic elaboration at encoding, reflected by differential activity in LIFG and/or MTL, and better memory performance during recognition for negative words, again paired to differential activity in LIFG and/or MTL. For neutral stimuli we did not expect to see an effect of the risk for anxiety and depression on either performance or brain activation and for positive stimuli we expected effects in the opposite direction. The concordant/ discordant twin design further enabled us to explore whether environmental and genetic risk factors for anxiety and depression influence performance and brain activation during verbal encoding and recognition in different ways.

Material and methods

Subjects

Selection of subjects has been described in detail by de Geus *et al.* (2007). Briefly, in a sample of 2455 same sex twin pairs registered in the Netherlands Twin Registry, a compound risk score for anxiety and depression was computed based on a genetic factor analysis of longitudinal survey data on trait anxiety, depression, neuroticism and somatic anxiety (for details see Boomsma *et al*., 2000). The surveys were collected in 1991, 1993, 1997 and 2000. An average risk score was computed for each twin across all available surveys (which could vary from one to four surveys).

Discordant MZ twin pairs were considered eligible for participation if both were right-handed, the high-risk subject had a risk score at least 0.5 standard deviation above the mean, and the score of high-and low-risk twins were at least 2 standard deviations apart. To estimate the relative risk for actual anxiety and depression disorder in twins ascertained by these criteria, we selected all subjects with comparable low-or high-risk scores from the larger sample of 1256 subjects that underwent a CIDI interview in 2000 (Middeldorp *et al*., 2004). To resemble the observed mean risk scores in our discordant twins (-0.62 vs. 0.97, S.D. = 0.74), we selected subjects with a risk score of at least 1.5 standard deviation above the mean (1.06) on the 1991, 1993, and 1997 surveys and subjects with a risk score of at least 0.5 standard deviations below the mean (-0.32). In this sample the relative risk to receive a lifetime depression diagnosis in the high-scoring subjects was 11.8 compared to lowscoring subjects and 40.1 for generalized anxiety disorder.

A total of 31 discordant MZ twin pairs met our criteria, of which 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 out of time constraints. One pair turned out to be dizygotic. This left a final 10 MZ pairs who were extremely discordant for the risk for anxiety and depression. Notably, the risk scores of the high-risk twin in these final 10 pairs did not differ from the risk scores in the high-risk twin from the original 31 selected pairs.

Concordant MZ twin pairs were considered eligible for participation if both were right-handed and both their risk scores were at least 0.8 standard deviation above (high risk) or below (low risk) the mean risk score. An even more stringent selection of extreme risk scores was possible here compared to the discordant MZ twin selection, because extreme scoring concordant MZ pairs are much less rare than extremely discordant pairs. This yielded 115 concordant high-risk and 137 concordant low-risk pairs, of which we invited 48 pairs that 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 refused to participate, mainly out of time constraints. This left a final 15 MZ pairs who were concordant for low risk and 7 MZ pairs who were concordant for high risk for anxiety and depression. Of the total group of 64 MZ, 28 were male and the mean age was 30 years (range 20–42 years).

Procedure

Subjects visited the outpatient MR unit and experimental procedures were explained in detail. Twins from the same pair always came on the same day. Twins were randomly assigned to an MRI scan session or a psychometric session. After about 90 min twins switched between sessions. During the psychometric session, cognitive abilities and current psychiatric diagnostic state were assessed. All subjects were interviewed using the Composite International Diagnostic Interview (Peters and Andrews, 1995; Wittchen, 1994), a clinical interview that assessed the occurrence of a current or recent depressive episode (6 months). The Montgomery Asberg Depression Rating Scale and the Beck Depression Inventory were used to assess depressive symptom characteristics and severity scores (Beck *et al*., 1961; Montgomery and Asberg, 1979). Furthermore, the state version of the State-Trait Anxiety Inventory (Spielberger *et al*., 1970) was administered pre-and post-scanning. Verbal comprehension (IQ) and working memory (forward and backward recall scores of digit scan) were assessed using subtests of the Wechsler Adult Intelligence Scale (Wechsler, 1997). Finally, social support was measured using the Duke-UNC questionnaire (Broadhead *et al*., 1988) and subjects were asked to recall the occurrence of 21 major life events. These included individual (e.g. maltreatment, disease, financial problems, job strain, relational problems) events as well as network-related events (e.g. disease or loss of close kin). Subjects were asked to locate these events in four temporal valences, i.e. whether they occurred the last 6 months, between 6 and 12 months, between 1 and 5 years or more than 5 years ago. For each event they indicated the impact on their lives on a 10-point visual analogue scale ranging from 'no impact' to 'extreme impact'.

During each MRI session, which lasted 45 min, subjects performed two tasks using emotionally relevant stimuli (faces and words). At first, a verbal memory task took place, followed by an emotional faces paradigm, results of which will be reported elsewhere. Between the encoding and recognition phase of the memory paradigm a structural MR scan was performed. At the end of both sessions subjects were debriefed and received sets of buccal swabs to collect mucosal cells for DNA extraction. DNA was used to confirm zygosity using 11 highly polymorphic markers. The ethical review board of the VU medical centre approved the study and all participants provided written informed consent.

Task paradigm

Prior to scanning, all participants practiced the event-related encoding paradigm outside the scanner on a personal computer. In the scanner, a device with response buttons was positioned near the right hand of the participant. During all task blocks, participants had to respond in a forced choice fashion by pushing the left button with the index finger or the middle button with the middle finger or the right button with the ring finger. Stimuli were presented in a self-paced fashion, although a time limit of 5 s was maintained in case of nonresponses. The event-related retrieval paradigm followed 15 min after the encoding phase and was explained and practiced inside the scanner and presented by surprise.

Participants were requested to judge the valence of the word during the encoding phase. During recognition, they were requested to indicate whether or not each word had appeared previously in the encoding task. On each trial, response options were indicated at the bottom of the screen by an arrow pointing to the left ('«« negative' in the encoding; '«« seen' in the retrieval), and middle ('»»neutral«« ', '»»probably seen«« ') and right ('positive»»'; 'not seen««'). Beforehand, it was explained that pushing the left button corresponded to the left arrow, etc. Scores were only registered when participants responded within the 5 s time limit. To increase experimental power and to minimize expectancy effects, a variable interstimulus interval (200–600 ms, offset to onset) was used.

Forty baseline trials were added to each phase (encoding and recognition). During a baseline trial, participants were presented with a cue to press either the left ('«« left') or the middle ('«« middle»»') or the right button ('right»»'). All types of baseline stimuli were presented equally often.

Material

The stimuli consisted of 240 Dutch words and 80 baseline stimuli. One third of the words had a negative connotation, one third of the words had a neutral connotation and one third of the words had a positive connotation. The valence of the stimulus material was validated in a perceptual clarification task (Ter Laak, 1992, unpublished Master's thesis), in which these words were recognized most consistently and rapidly as negative, neutral and positive words under conditions of minimal presentation conditions. A subset of 40 positive, 40 neutral and 40 negative words were randomly selected and ordered for the affective evaluation task (encoding). All three subsets were matched for word length, word type (verbs, adjectives and nouns) and frequency of usage. The use of abstract words was avoided. For the recognition phase, another subset of 40 positive, 40 neutral and 40 negative words were randomly selected in the same way and designated as 'new' words.

Before the experiment, the trials were randomly intermixed into blocks. For the encoding phase, 20 blocks consisting of 2 positive, 2 negative, 2 neutral words and 2 baselines were

randomised. The resulting random sequence of 160 stimuli was used for all subjects to optimise comparison between subjects. For the recognition phase 20 blocks consisting of 2 positive, 2 neutral and 2 negative words from the encoding phase and 2 positive, 2 neutral and 2 negative new words and 2 baselines were randomised. Half of the words were 'new' words and half were old words presented during the encoding phase. As for encoding, the same random sequence of 280 stimuli was generated once and then used for all subjects. To prevent primacy and recency effects in the recognition task, three buffer words proceeded and followed the encoding stimuli. In addition, to let the participants get used to the unexpected recognition task, three buffer words preceded this phase. No feedback regarding the answers was provided during the experiment.

Performance measures

Performance during the encoding phase was measured by the error rates and mean reaction times (RT) for words that were later correctly recognized as 'seen' with certainty. In the recognition phase, sensitivity (Pr = proportion hits -proportion false alarms) and response bias Br (=false alarms/(1 -Pr)) were calculated for encoded negative, neutral and positive items, according to two-high-threshold theory (Snodgrass and Corwin, 1988).

Image acquisition

Magnetic resonance imaging was performed on a 1.5-T Sonata MR system (Siemens, Erlangen, Germany) with a standard RF receiver head coil. Stimuli were generated by a Pentium PC and projected on a screen at the end of the scanner table, which was seen through a mirror mounted above the subject's head. Two magnet-compatible four-key response boxes were used to record subject's performance and reaction times (RTs). To reduce motion artefacts, the subject's head was immobilized using foam pads.

For functional MRI, an echo planar imaging sequence (TR 3.04 s, TE 45 ms, matrix 64 x 64, field of view 192 mm x 192 mm, flip angle 90°) was used, creating transversal whole-brain acquisitions (35 slices, 3 x 3-mm in-plane resolution, 2.5-mm slice thickness, 0.5-mm interslice gap).

Data analysis

Differences in the questionnaire-and interview-based variables were examined by a mixed model ANOVA (MIXED SPSS) with group (discordant low risk, discordant high risk, concordant low risk, concordant high risk) as a fixed factor and family as a random factor to account for within-family dependence. With respect to performance, error rates and mean reaction times (RT) for correct responses during encoding and hits and false alarms as well as the two signal detection measures during recognition were tested by a similar MIXED ANOVA that additionally added word valence (negative, neutral and positive) as a second fixed factor. Primary planned contrasts in main or interaction effects involving groups were the comparison of the low-risk twin versus the high-risk co-twin in discordant pairs and the concordant low-risk twin pairs versus the concordant high-risk twins.

Imaging data were analysed using SPM2 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK). After the first two volumes were discarded to allow for magnetic saturation, time series were corrected for differences in slice acquisition times and realigned. Next, data were warped to MNI space as defined by the SPM EPI template, and spatially smoothed using an 8-mm FWHM Gaussian kernel. After spatial preprocessing, data were analysed using delta functions convolved with a canonical hemodynamic response function to model responses to each stimulus type. For each subject, weighted contrasts were computed for 4 simple main effects: this was done across all word valences (correctly remembered words vs. baseline), and for each word valence separately (correctly remembered positive/negative/ neutral words vs. baseline). The resulting contrast images were entered into second-level (random effects) analyses for between subject comparisons. A paired *t*-test was used for the comparison of the low-risk twin versus the highrisk co-twin in discordant pairs (Twin) and a one-way ANOVA for the comparison of concordant low-and high-risk pairs (Group). SPM2's nonsphericity option was used in the latter analysis to account for within-pair correlated observations. Main effects of all words across groups are reported at $p<0.05$ FDR-corrected for multiple comparisons with an extent threshold of five voxels (Genovese *et al.*, 2002). Group interactions across word valence and interactive effects of Group/Twin by Word valence (negative vs. baseline, neutral vs. baseline, positive vs. baseline; masked with the relevant main effect) are reported at $p<0.001$ uncorrected, unless indicated otherwise, also with an extent threshold of five voxels.

Results

Demographic data and depression/anxiety ratings (BDI, MADRS, STAI-state) obtained at the time of the scanning session are listed in Table 1. Age and male/female distribution were not significantly different across the groups. Mixed ANOVA confirmed that the concordant low-risk pairs scored significantly lower on the MADRS, BDI, and state anxiety measures than the concordant high-risk pairs. Within the discordant pairs, the MADRS, BDI, and state anxiety measures all showed significant intra-pair differences in the expected direction. Only one subject in a concordant high-risk pair received a current diagnosis of depression using these instruments. Furthermore, one subject currently used antidepressant medication (SSRI). This was the subject with the high anxious depression score from a discordant pair. These two subjects were excluded from all further analyses. Data from one concordant low-risk pair were lost due to technical problems during scanning.

Behavioral data

Encoding phase (affective classification)

Across all four groups (concordant low risk, concordant high risk, discordant low risk, and discordant high risk) reaction times for words later correctly recognized with certainty were fastest for negative words (mean reaction time negative words: 1106 ± 251 ms vs. neutral words: 1332 ± 345 ms and positive words: 1235 ± 331 ms; $F(2, 127.3) = 16.15$, $p<0.001$). Error rate for the affective classification of successfully recognized words was also lower for negative words (negative words: 9% vs. neutral words: 27% and positive words: 21%; $F(2, 121.8) = 26.3$, $p<0.001$). Importantly, no main group or group x valence interaction effects were found suggesting that affective classification does not differ as a function of the risk for anxiety and depression.

Recognition phase (retrieval)

Performance data were missing during retrieval for four subjects and these were excluded from further analyses of the performance data. Hit rates, false alarm rates (FA), sensitivity (Pr) scores and response bias (Br) for the remaining 56 subjects are shown in Table 2. Valence had a significant effect on the number of hits ($F(2, 125.4) = 3.39$, $p = 0.039$), false alarms ($F(2, 126.0) = 25.2$, $p<0.001$), sensitivity ($F(2, 126.5) = 7.1$, $p = 0.001$), and Br ($F(2, 124.6) = 22.8$, $p<0.001$). More hits as well as false alarms were made on positive and negative words than on neutral trials, and neutral trials had a smaller response bias. These effects were due to the fact that neutral trials were recognized with certainty much less often than positive or negative words. Sensitivity, however, was significantly lower for negative words than for either neutral or positive words.

Table 1: Characteristics of the twins at the time of MRI scanning

	Low risk concordant twins (N = 30)	Discordant twin pairs		High risk concordant twins (N = 14)
		Low risk twin (N = 10)	High risk twin (N = 10)	
Male/female, no.	14/16	4/6	4/6	6/8
Age, mean (year)	30.9	30.6	30.6	26.1
BDI depression, mean (S.D.)[a,b]	1.1 (1.5)	2.7 (2.5)	9.7 (10.6)	8.0 (6.0)
MADRS, mean (S.D.)[a,b]	0.43 (1.5)	2.1 (2.5)	5.0 (3.9)	5.1 (7.8)
STAI state anxiety before scan session, mean (S.D.)[a,b]	27.7 (5.0)	31.4 (5.3)	37.0 (7.3)	36.7 (9.8)
STAI state anxiety after scan session, mean (S.D[a,b]	25.1 (4.7)	28.3 (5.5)	37.1 (8.1)	33.8 (8.4)
Verbal IQ subscale, mean (S.D.)	13.1 (2.5)	14.5 (2.2)	14.6 (2.2)	12.9 (3.9)
Working memory IQ subscale, mean (S.D.)	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 intra-pair difference in discordant twins.

A main effect of group was found on sensitivity only ($F(3, 37.8) = 6.2$, $p = 0.002$), which did not interact with valence. Post hoc comparisons revealed that, across all word valences, a significant difference between the high-risk twin and the low risk twin from discordant pairs was found ($p<0.001$). A trend in a similar direction was seen when comparing the concordant high-risk pairs with the concordant low-risk pairs ($p = 0.079$). In all instances the high-risk subjects performed better. Across all word valences they had higher hit rates and lower false alarm rates than the low-risk subjects resulting in higher sensitivity scores.

Imaging data

Results of random effects (RFX) analyses of the imaging data are summarized in Tables 3–5 and are shown in 3D visualizations and cross sections in Figs. 1 and 2.

Encoding phase

Fig. 1 shows the average BOLD effects across all 4 groups when comparing all correctly encoded words against baseline stimuli (glass brain).

Across groups, emotional classification of words compared to baseline was associated with robustly increased blood oxygenation dependent (BOLD) signal in left inferior frontal gyrus (LIFG). Furthermore, regions showing increased activity were bilateral hippocampus (HC), as well as dorsal anterior cingulate cortex (ACC), and visual processing areas, including bilateral occipital cortex and left fusiform gyrus, situated in the ventral route (cf. Table 3).

Modulation by the risk for anxiety and depression

Across word valences, we observed increased activity in LIFG (45, 42, 6; Z = 3.34) in the concordant high-risk group compared to the concordant low-risk group. Conversely, decreased left hippocampal activity was found in concordant high-risk group compared to concordant low-risk group (Z = 3.15). Across word valences, we did not find significant differences between high-risk discordant twins and their low-risk co-twins. Interaction analyses by word valence (negative, positive and neutral; Table 4) showed increased activation during encoding of negative words in concordant high-risk twins in left occipital cortex (Z = 3.20). Conversely, decreased activation in left hippocampus was found. During encoding of neutral words, concordant high-risk twins showed increased activation in occipital cortex and left fusiform gyrus compared to concordant low-risk twins. At a slightly lower threshold (p = 0.003, Z = 2.71), more LIFG activation was also found in these highrisk twins. Interaction analyses of encoding of positive words showed increased activation in concordant high-risk twins in the LIFG and right amygdala. In discordant twins, only one significant group by word valence interaction was found: for negative words more right amygdala was found in the high-risk twin compared to the low-risk co-twin (Z = 3.22).

Table 2: Mean proportions (S.D. in parentheses) of hit rates, false alarm rates (FA), sensitiviy (Pr) and response bias (Br) across all valences and groups and separately per group and word valence

	Concordant twins		Discordant twins		All groups
	Low risk	High risk	Low risk	High risk	
Hits					
Negative	0.72 (0.12)	0.74 (0.10)	0.72 (0.23)	0.73 (0.13)	0.72 (0.14)
Neutral	0.59 (0.19)	0.73 (0.15)	0.67 (0.16)	0.74 (0.15)	0.66 (0.17)
Positive	0.67 (0.17)	0.79 (0.09)	0.73 (0.23)	0.78 (0.10)	0.73 (0.16)
All words	0.66 (0.14)	0.75 (0.09)	0.71 (0.18)	0.75 (0.09)	0.71 (0.14)
FA					
Negative	0.23 (0.16)	0.20 (0.13)	0.22 (0.18)	0.16 (0.12)	0.21 (0.15)
Neutral	0.09 (0.07)	0.07 (0.06)	0.12 (0.16)	0.06 (0.04)	0.09 (0.09)
Positive	0.18 (0.14)	0.18 (0.11)	0.18 (0.22)	0.11 (0.07)	0.17 (0.15)
All words	0.17 (0.11)	0.15 (0.09)	0.17 (0.18)	0.11 (0.07)	0.15 (0.12)
Pr					
Negative	0.49 (0.17)	0.54 (0.16)	0.50 (0.17)	0.57 (0.14)	0.51 (0.16)
Neutral	0.50 (0.18)	0.66 (0.14)	0.55 (0.21)	0.68 (0.18)	0.58 (0.19)
Positive	0.49 (0.19)	0.62 (0.15)	0.56 (0.23)	0.67 (0.15)	0.56 (0.19)
All words	0.49 (0.17)	0.61 (0.13)	0.54 (0.18)	0.64 (0.14)	0.55 (0.16)
Br					
Negative	0.43 (0.21)	0.41 (0.18)	0.45 (0.32)	0.36 (0.20)	0.42 (0.22)
Neutral	0.20 (0.15)	0.23 (0.23)	0.22 (0.22)	0.18 (0.11)	0.21 (0.18)
Positive	0.35 (0.22)	0.45 (0.22)	0.39 (0.31)	0.31 (0.12)	0.37 (0.23)
All words	0.33 (0.16)	0.36 (0.17)	0.35 (0.27)	0.28 (0.10)	0.33 (0.18)

Table 3: Encoding: BOLD main effects for all correctly encoded words vs. baseline across groups at p < 0.05, FDR corrected for multiple comparisons

Region	*x*	*y*	*z*	Z value
Prefrontal				
Lateral inferior				
L	- 48	27	3	6.82
L	- 51	30	- 6	5.94
L	- 42	33	- 3	4.86
Medial				
L	- 18	54	30	3.31
Anterior cingulate				
L	- 6	21	45	4.13
Medial temporal lobe				
R	21	- 12	- 21	5.04
L	- 21	- 24	- 6	4.11
L	- 18	- 15	- 21	3.66
L	- 27	- 12	- 21	3.46
Occipital				
R	15	- 99	12	6.21
L	- 15	- 90	9	6.02
R	6	- 96	6	5.81

Table 4: Encoding: BOLD group interactions for word valences at p < 0.001 uncorrected

	Negative vs. baseline					Positive vs. baseline					Neutral vs. baseline				
	L/R	*x*	*y*	*z*	Z value	L/R	*x*	*y*	*z*	Z value	L/R	*x*	*y*	*z*	Z value
LC > HC															
Medial temporal	L	- 21	- 24	- 18	3.09										
HC > LC															
Prefrontal															
Lateral inferior						L	- 45	42	6	3.78					
						L	- 42	36	- 3	3.24					
Medial temporal											L	- 15	- 42	- 3	3.61
Occipital	L	- 15	- 60	3	3.20*	L	- 27	- 96	18	3.55	R	9	- 93	9	3.72
						L	- 33	- 72	- 9	3.62					
											R	15	- 63	- 9	3.33
											R	39	- 90	0	3.21
HD > LD															
Amygdala	R	27	- 3	- 18	3.22*										

HC = high concordant; LC = low concordant; HD = high discordant; LD = low discordant.

Recognition phase

Fig. 2 depicts activation for all groups of the cortical network in response to correct recognition and correct rejection of encoded words across valences. Main regions for this comparison are listed in Table 5. Activity was left lateralized, like in the encoding task, although right hemisphere activation was more apparent in the recognition phase. We found the activation of the LIFG, dorsal ACC, and bilateral occipital cortex, areas that were also active during encoding. In addition, we found bilateral insular cortex activation.

*Modulation by the risk for anxiety and depressio*n

Across word valences, concordant high-risk twins showed increased LIFG compared to concordant low-risk twins (Z = 3.41), whereas in discordant twins we failed to observe significant effects of risk status. Interaction analyses with valence showed that compared to concordant low-risk twins, concordant high-risk twins showed increased activation in the LIFG only during negative words (Table 6). In addition, during retrieval of negative words increased LIFG and RIFG activation was found in discordant high-risk twins compared to their discordant co-twin albeit LIFG at a lower statistical level (Table 6).

Table 5: Retrieval: BOLD main effects for all correctly recognized/rejected words vs. baseline across groups at $p < 0.05$, FDR corrected for multiple comparisons

Region	*x*	*y*	*z*	Z value
Prefrontal				
Lateral inferior				
L	- 45	27	21	4.05
L	- 57	30	24	3.41
Anterior cingulate				
R	6	27	42	3.32
Insula				
L	- 30	21	- 3	4.59
R	30	24	- 3	5.14
Occipital				
L	- 39	- 78	- 12	5.91
L	- 24	- 93	12	5.63

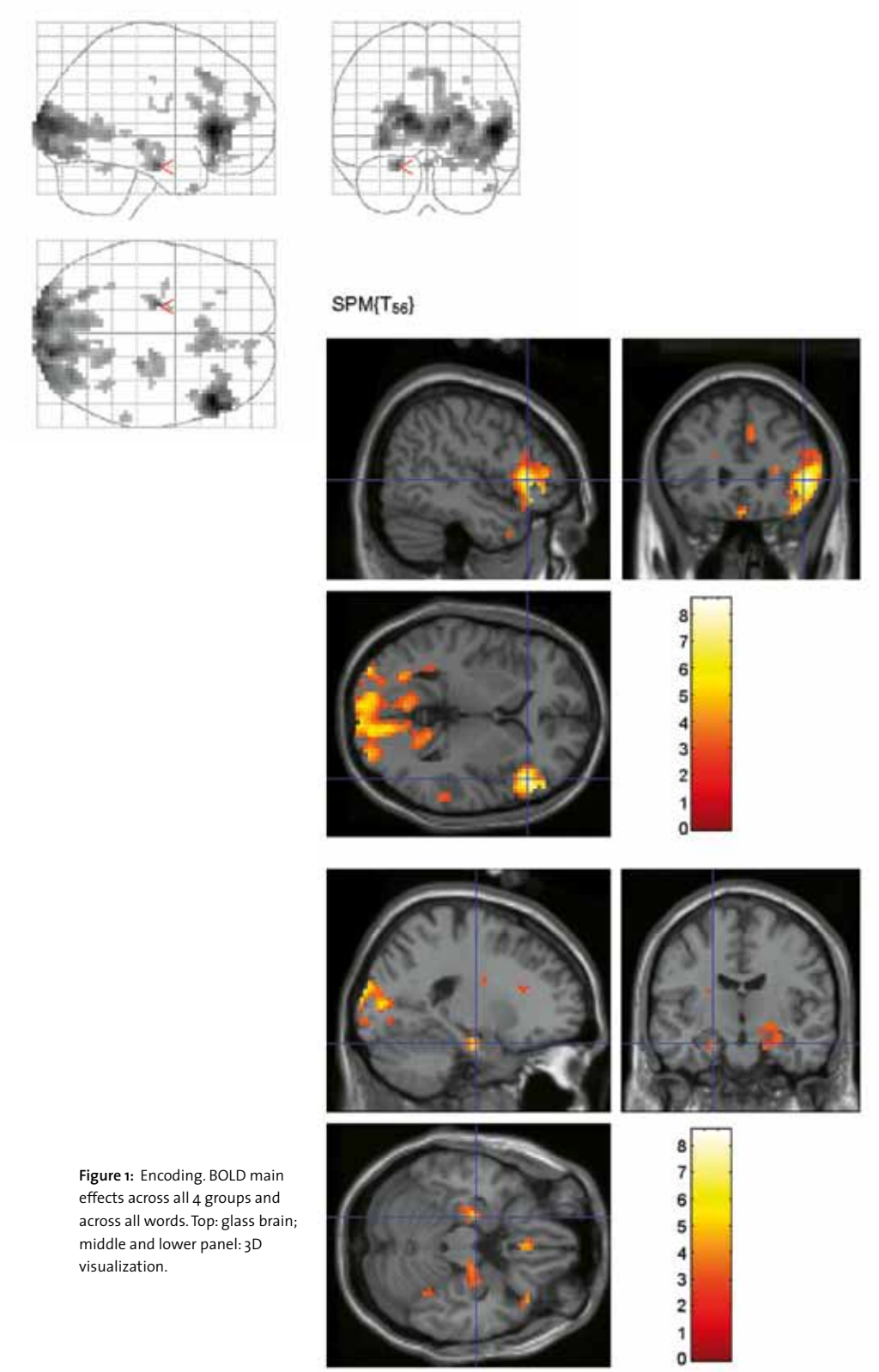

Figure 1: Encoding. BOLD main effects across all 4 groups and across all words. Top: glass brain; middle and lower panel: 3D visualization.

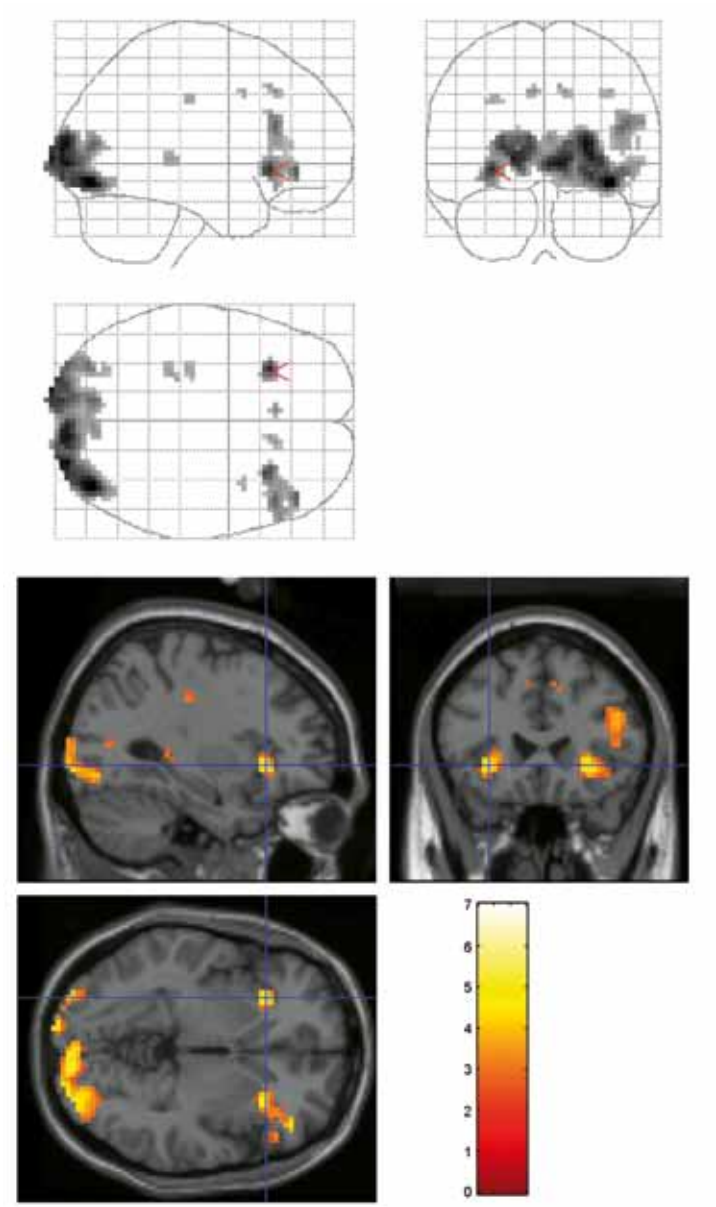

Figure 2: Retrieval. BOLD main effect for all words across groups. Top: glass brain and lower panel: 3D visualization.

Table 6: Retrieval: BOLD group interactions for word valences at $p < 0.001$ uncorrected

	Negative vs. baseline					Positive vs. baseline					Neutral vs. baseline				
	L/R	*x*	*y*	*z*	Z value	L/R	*x*	*y*	*z*	Z value	L/R	*x*	*y*	*z*	Z value
HC > LC															
Prefrontal															
Lateral inferior	L	- 42	42	0	3.05*										
Insula											L	- 36	33	0	3.90
HD > LD															
Prefrontal															
Lateral inferior	L	- 54	33	6	2.65#										
	R	48	36	18	3.83										
Hippocampus											L	- 36	- 21	- 21	3.37

HC = high concordant; LC = low concordant; HD = high discordant; LD = low discordant.
# $p = 0.004$.

Discussion

The goal of the present study was to investigate possible emotional biases in subjects who were at risk for anxiety and depression through either genetic or environmental factors, but not yet clinically affected. To this end, we employed an incidental encoding task followed by an explicit retrieval task for emotional versus neutral verbal information in monozygotic twins, that were either strongly concordant or very discordant for scores on neuroticism, anxiety, and depression in longitudinal surveys. We expected that the high-risk twins would be characterized by differential encoding and retrieval of emotional words, particularly negative words, both on a behavioral and a neural level.

In contrast to our expectation, behavioral data acquired during the encoding phase did not show differences between high-risk and low-risk twins. During recognition, we did observe improved memory performance in high-risk subjects compared to low-risk subjects, but this was true across all types of words, and not specific for negative words. Therefore, our behavioral data do not support the hypothesis that a negative response bias may be present in subjects at risk for depression/ anxiety. This was true for subjects at risk through environmental factors (discordant pairs) as well through genetic factors (concordant pairs). Our findings are at odds with previous studies showing increased recall of recently learned negatively valence words (Rinck and Becker, 2005) and decreased recognition of happy facial expressions (Gotlib *et al.*, 2004; Surguladze *et al.*, 2004) in depressed patients. These negative biases were not simply due to depressive state at the time of scanning, as they

tended to persist in patients recovered from clinical depression (Leppanen, 2006). Also, a recent study (Joormann *et al.*, 2007) showed a clear negative bias in a dot probe task in young people at risk for depression through having a depressed mother, but not yet depressed. This latter study, however, employed a negative mood induction paradigm. Negative perceptual and memory biases following negative mood induction have also been found in healthy volunteers without a family history (Bouhuys *et al.*, 1995).

Results of other studies do converge with ours. A recent study in non-depressed people at familiar risk for depression failed to reveal evidence for a negative emotional bias in either a facial expression or an emotion categorization task (Mannie et al., 2007). With regard to memory performance, Pine *et al.* (2004) did not find altered recall of emotional expressions in unaffected offspring of depressed parents. Furthermore, in a recent study in elderly non-depressed first-degree relatives of patients with major depression compared to controls, no evidence was found for a negative bias in facial expression recognition accuracy or word recall (Le Masurier *et al.*, 2007). In this study, the depressed relative group responded faster during recognition of facial expressions of fear, but not for anger, disgust and sad faces. In addition, relatives responded slower when they had to recognize positive personality characteristics in a verbal categorization task, but there were no differences for negative words between groups (Le Masurier *et al.*, 2007).

Taking together findings from the above studies with the present findings, we are led to conclude that subjects at risk for depression, either through genetic or environmental determinants, do not exhibit a negative emotional bias at the behavioral level, unless emotional material is presented immediately after negative mood induction. Only in a setting of lowered mood vulnerable individuals might respond with larger negative emotional biases. This would potentially place them at greater risk of experiencing more severe or prolonged dysphoric reactions in response to negative stimuli (Mannie *et al.*, 2007). The above conclusion, however, is based only on the behavioral results and needs to be interpreted against the background of parallel responses at the neural level.

For BOLD activation we also hypothesized differential results for low-and high-risk subjects, which we expected to be most prominent during negative emotional words. Overall, imaging results were in line with previous studies using this paradigm. During encoding, emotional classification of words compared to baseline was associated with a robustly increased BOLD signal in the dorsal ACC and the LIFG, the latter reflecting semantic elaboration of words. Furthermore, regions showing increased activity were the bilateral occipital cortex, bilateral hippocampal region, and the left posterior medial temporal cortex, probably reflecting the visual analysis of written word forms (orthographic processing). During explicit retrieval in the recognition phase we observed similar brain regions as in the encoding phase, although activity tended to be more bilateral compared

to the relative left lateralization during encoding, and MTL activity was absent. These areas correspond closely to those reported in other fMRI studies of previously in fMRI studies using a similar paradigm (Daselaar lexical decision tasks indicating the involvement of neural *et al.*, 2004; de Ruiter et al., 2007), other investigators have circuits responsible for semantic processing (Jobard *et al.*, failed to detect significant MTL activity. Therefore, it has been 2003) and verbal declarative memory (Buckner and Koutstaal, suggested that the hippocampus is mainly involved in 1998). Although hippocampal activity has been reported associative memory rather than single-item memory (as in our task), and that hippocampal activity observed during singleitem memory tasks is due to e.g. novelty effects (Squire et al., 2004, for an overview). The issue is still undecided, as a recent study demonstrated that hippocampal damage may impair both associative and single-item memory (Gold *et al.*, 2006), and that familiarity-based recognition for complex visual stimuli is supported by the hippocampus, as demonstrated by single-cell recordings (Rutishauser *et al.*, 2006). Our data generally support the hypothesis that the hippocampus is involved during successful encoding of single items (words).

Our main interest was the modulation of activity in these areas by genetic and environmental risk for anxiety and depression. In contrast to analyses at the behavioral level we found clear evidence that brain activation during encoding and retrieval of emotional stimuli is indeed influenced by these risk factors. During encoding, we found increased activity in the LIFG in concordant high-risk twins compared to the concordant low-risk twins across all word valences. High-and low-risk individuals from discordant twin pairs did not show this difference. During recognition we again found increased activity in the LIFG in high-risk twins, this time in both discordant and concordant pairs. A parsimonious explanation for the increased LIFG activation in high-risk subjects is that it reflects their better performance during the recognition task. Previous studies in healthy volunteers have indicated that greater activation is found in left inferior frontal and fusiform regions for remembered than forgotten words (Baker *et al.*, 2001). Importantly, however, the effect of risk on LIFG activation, but not on performance, was most evident in negatively valenced words. Therefore, our findings could indicate that high-risk subjects engage in increased elaboration of negative words compared to the low-risk subjects. Further evidence for differential effects of negative words on the low-and high-risk subjects was found in the amygdala response during encoding. In keeping with studies showing larger reactivity to negative emotional faces (Canli *et al.*, 2005; Fu *et al.*, 2004; Siegle *et al.*, 2001; Surguladze *et al.*, 2005) discordant high-risk twins showed larger right amygdala activation to negative words than their low-risk co-twin.

In conclusion, in the present fMRI study we failed to find evidence at the behavioral level for a negative response bias in subjects at high risk for anxiety and depression. At the neural

level, increased LIFG activation by negative words was found in high-risk subjects, most prominently during retrieval. These fMRI results applied to subjects at high risk through either genetic or environmental risk factors. They suggest that fMRI activation of the LIFG in a verbal emotional memory task may be a useful vulnerability marker for anxiety and depression.

References

1. Andrews, G., Peters, L., 1998. The psychometric properties of the Composite International Diagnostic Interview. Social Psychiatry and Psychiatric Epidemiology 33, 80–88.
2. Baker, J.T., Sanders, A.L., Maccotta, L., Buckner, R.L., 2001. Neural correlates of verbal memory encoding during semantic and structural processing tasks. Neuroreport 12, 1251–1256.
3. Beck, A.T., 1967. Depression: Causes and Treatment. University of Pennsylvania Press, Philadelphia.
4. Beck, A.T., Erbaugh, J., Ward, C.H., Mock, J., Mendelsohn, M., 1961. An inventory for measuring depression. Archives of General Psychiatry 4, 561–571.
5. Beevers, C.G., Wells, T.T., Miller, I.W., 2007. Predicting response to depression treatment: the role of negative cognition. Journal of Consulting and Clinical Psychology 75, 422–431.
6. Bhagwagar, Z., Cowen, P.J., Goodwin, G.M., Harmer, C.J., 2004. Normalization of enhanced fear recognition by acute SSRI treatment in subjects with a previous history of depression. American Journal of Psychiatry 161, 166–168.
7. Boomsma, D., Busjahn, A., Peltonen, L., 2002. Classical twin studies and beyond. Nature Reviews Genetics 3, 872–882.
8. Boomsma, D.I., Beem, A.L., van den, B.M., Dolan, C.V., Koopmans, J.R., Vink, J.M., *et al.*, 2000. Netherlands twin family study of anxious depression (NETSAD). Twin Research 3, 323–334.
9. Bouhuys, A.L., Bloem, G.M., Groothuis, T.G.G., 1995. Induction of depressed and elated mood by music influences the perception of facial emotional expressions in healthy-subjects. Journal of Affective Disorders 33, 215– 226.
10. Bradley, B.P., Mogg, K., Williams, R., 1995. Implicit and explicit memory for emotion-congruent information in clinical depression and anxiety. Behaviour Research and Therapy 33, 755–770.
11. Broadhead, W.E., Gehlbach, S.H., Degruy, F.V., Kaplan, B.H., 1988. The Duke-Unc Functional Social Support Questionnaire—measurement of social support in family medicine patients. Medical Care 26, 709–723.
12. Buckner, R.L., Koutstaal, W., 1998. Functional neuroimaging studies of encoding, priming, and explicit memory retrieval. Proceedings of the National Academy of Sciences of the United States of America 95, 891–898.
13. Canli, T., Cooney, R.E., Goldin, P., Shah, M., Sivers, H., Thomason, M.E., *et al.*, 2005. Amygdala reactivity to emotional faces predicts improvement in major depression. Neuroreport 16, 1267–1270.

14. Caspi, A., Taylor, A., Moffitt, T.E., Plomin, R., 2000. Neighborhood deprivation affects children's mental health: environmental risks identified in a genetic design. Psychological Science 11, 338–342.
15. Daselaar, S.M., Veltman, D.J., Witter, M.P., 2004. Common pathway in the medial temporal lobe for storage and recovery of words as revealed by event-related functional MRI. Hippocampus 14, 163–169.
16. de Geus, E.J., Ent, D.V., Wolfensberger, S.P., Heutink, P., Hoogendijk, W.J., Boomsma, D.I., *et al.*, 2007. Intrapair differences in hippocampal volume in monozygotic twins discordant for the risk for anxiety and depression. Biological Psychiatry 61, 1062–1071.
17. de Ruiter, M.B., Veltman, D.J., Phaf, R.H., van Dyck, R., 2007. Negative words enhance recognition in nonclinical high dissociators: an fMRI study. Neuroimage 37, 323–334.
18. Eley,T.C., Sugden,K., Corsico, A.,Gregory,A.M., Sham,P., McGuffin, P., *et al.*, 2004. Gene-environment interaction analysis of serotonin system markers with adolescent depression. Molecular Psychiatry 9, 908–915.
19. Fletcher, P.C., Henson, R.N.A., 2001. Frontal lobes and human memory— insights from functional neuroimaging. Brain 124, 849–881.
20. Fu, C.H.Y., Williams, S.C.R., Cleare, A.J., Brammer, M.J., Walsh, N.D., Kim, J., *et al.*, 2004. Attenuation of the neural response to sad faces in major depression by antidepressant treatment—a prospective, event-related functional magnetic resonance imaging study. Archives of General Psychiatry 61, 877–889.
21. Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 15, 870–878.
22. Gold, J.J., Smith, C.N., Bayley, P.J., Shrager, Y., Brewer, J.B., Stark, C.E., *et al.*, 2006. Item memory, source memory, and the medial temporal lobe: concordant findings from fMRI and memory-impaired patients. Proceedings of the National Academy of Sciences of the United States of America 103, 9351–9356.
23. Gotlib, I.H., Kasch, K.L., Traill, S., Joormann, J., Arnow, B.A., Johnson, S.L., 2004. Coherence and specificity of information-processing biases in depression and social phobia. Journal of Abnormal Psychology 113, 386–398.
24. Grabe, H.J., Lange, M., Wolff, B., Volzke, H., Lucht, M., Freyberger, H.J., *et al.*, 2005. Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden. Molecular Psychiatry 10, 220–224.
24. Gur, R.C., Erwin, R.J., Gur, R.E., Zwil, A.S., Heimberg, C., Kraemer, H.C., 1992. Facial emotion discrimination. 2. Behavioral findings in depression. Psychiatry Research 42, 241–251.
25. Hayward, G., Goodwin, G.M., Cowen, P.J., Harmer, C.J., 2005. Low-dose tryptophan depletion in recovered depressed patients induces changes in cognitive processing without depressive symptoms. Biological Psychiatry 57, 517–524.

26.Jobard, G., Crivello, F., Tzourio-Mazoyer, N., 2003. Evaluation of the dual route theory of reading: a metanalysis of 35 neuroimaging studies. Neuroimage 20, 693–712.
27.Joormann, J., Talbot, L., Gotlib, I.H., 2007. Biased processing of emotional information in girls at risk for depression. Journal of Abnormal Psychology 116, 135–143.
28.Kaufman, J., Yang, B.Z., Douglas-Palumberi, H., Grasso, D., Lipschitz, D., Houshyar, S., *et al.*, 2006. Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. Biological Psychiatry 59, 673–680.
29.Kendler, K.S., Heath, A., Martin, N.G., Eaves, L.J., 1986. Symptoms of anxiety and depression in a volunteer twin population—the etiologic role of genetic and environmental-factors. Archives of General Psychiatry 43, 213–221.
30.Kendler, K.S., Prescott, C.A., 1999. A population-based twin study of lifetime major depression in men and women. Archives of General Psychiatry 56, 39–44.
31.Le Masurier, M., Cowen, P.J., Harmer, C.J., 2007. Emotional bias and waking salivary cortisol in relatives of patients with major depression. Psychological Medicine 37, 403–410.
32.Leppanen, J.M., 2006. Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. Current Opinion in Psychiatry 19, 34–39.
33.Mannie, Z.N., Harmer, C.J., Cowen, P.J., 2007. Increased waking salivary cortisol levels in young people at familial risk of depression. American Journal of Psychiatry 164, 617–621.
34.Middeldorp, C.M., Cath, D.C., Beem, A.L., Boomsma, D.I., 2004. Genetic epidemiology of depression in a selected population of Dutch twins and their siblings. Behavior Genetics 34, 652–653.
35.Montgomery, S.A., Asberg, M., 1979. New depression scale designed to be sensitive to change. British Journal of Psychiatry 134, 382–389.
36.Murphy, B.C., Shepard, S.A., Eisenberg, N., Fabes, R.A., Guthrie, I.K., 1999. Contemporaneous and longitudinal relations of dispositional sympathy to emotionality, regulation, and social functioning. Journal of Early Adolescence 19, 66–97.
37.Peters, L., Andrews, G., 1995. Procedural validity of the computerized version of the Composite International Diagnostic Interview (Cidi-Auto) in the anxiety disorders. Psychological Medicine 25, 1269–1280.
38. Phillips, M.L., Drevets, W.C., Rauch, S.L., Lane, R., 2003. Neurobiology of emotion perception. II. Implications for major psychiatric disorders. Biological Psychiatry 54, 515–528.
39.Pine, D.S., Lissek, S., Klein, R.G., Mannuzza, S., Moulton, J.L., Guardino, M., *et al.*, 2004. Face-memory and emotion: associations with major depression in children and adolescents. Journal of Child Psychology and Psychiatry 45, 1199–1208.

40.Rinck, M., Becker, E.S., 2005. A comparison of attentional biases and memory biases in women with social phobia and major depression. Journal of Abnormal Psychology 114, 62–74.
41.Rutishauser, U., Mamelak, A.N., Schuman, E.M., 2006. Single-trial learning of novel stimuli by individual neurons of the human hippocampus-amygdala complex. Neuron 49, 805–813.
42.Siegle, G.J., Granholm, E., Ingram, R.E., Matt, G.E., 2001. Pupillary and reaction time measures of sustained processing of negative information in depression. Biological Psychiatry 49, 624–636.
43.Snodgrass, J.G., Corwin, J., 1988. Pragmatics of measuring recognition memory—applications to dementia and amnesia. Journal of Experimental Psychology-General 117, 34–50.
44.Spielberger, C.D., Gorsuch, R.L., Lushene, R.E., 1970. STAI Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA.
45. Squire, L.R., Knowlton, B.J., 2000. The medial temporal lobe, the hippocampus and the memory systems of the brain. In: Gazzaniga, M.S. (Ed.), The New Cognitive Neurosciences. The MIT Press, Cambridge, MA, pp. 765–779.
46.Squire, L.R., Stark, C.E.L., Clark, R.E., 2004. The medial temporal lobe. Annual Review of Neuroscience 27, 279–306.
47.Sullivan, P.F., Neale, M.C., Kendler, K.S., 2000. Genetic epidemiology of major depression: review and meta-analysis. American Journal of Psychiatry 157, 1552–1562.
48.Surguladze, S.A., Young, A.W., Senior, C., Brebion, G., Travis, M.J., Phillips, M.L., 2004. Recognition accuracy and response bias to happy and sad facial expressions in patients with major depression. Neuropsychology 18, 212–218.
49.Surguladze, S., Brammer, M.J., Keedwell, P., Giampietro, V., Young, A.W., Travis, M.J., *et al.*, 2005. A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. Biological Psychiatry 57, 201–209.
50.Teasdale, J.D., Russell, M.L., 1983. Differential-effects of induced mood on the recall of positive, negative and neutral words. British Journal of Clinical Psychology 22, 163–171.
51. Wechsler, D., 1997. WAIS-III Wechsler Adult Intelligence Scale. Psychological Corporation, San Antonio, Texas.
52. Wittchen, H.U., 1994. Reliability and validity studies of the who Composite International Diagnostic Interview (Cidi)—a critical-review. Journal of Psychiatric Research 28, 57–84.

SECTION B

Structural neuroimaging

4

Intrapair differences in hippocampal volume in monozygotic twins discordant for the risk for anxiety and depression

de Geus EJ, van't Ent D, Wolfensberger SP, Heutink P, Hoogendijk WJ, Boomsma DI, Veltman DJ.

Biological Psychiatry. 2007 May 1;61(9):1062-71. Epub 2006 Nov 29

Abstract

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.

Introduction

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).

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 ($r > 0.85$; 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 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 0.5 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 0.8 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 workingmemory 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 gradientecho T1-weighted sequence (magnetization prepared rapid gradient echo [MPRAGE]; sinversion time: 300 ms, time to repetition (TR) = 15 msec; echo time (TE) = 7 ms; flip angle = 8°; 160 slices, 1 x 1 x 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 0.05 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-byvoxel 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 0.05, 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<0.001, 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 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 nonnormalized 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).

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: Low Risk Twin	Discordant Twin pairs: High Risk Twin	High Risk Concordant Twins	
	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.

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.

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 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 voxelcluster 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

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

	Low Risk Concordant Twins	Discordant Twin pairs: Low Risk Twin	Discordant Twin pairs: High Risk Twin	High Risk Concordant Twins	
	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.

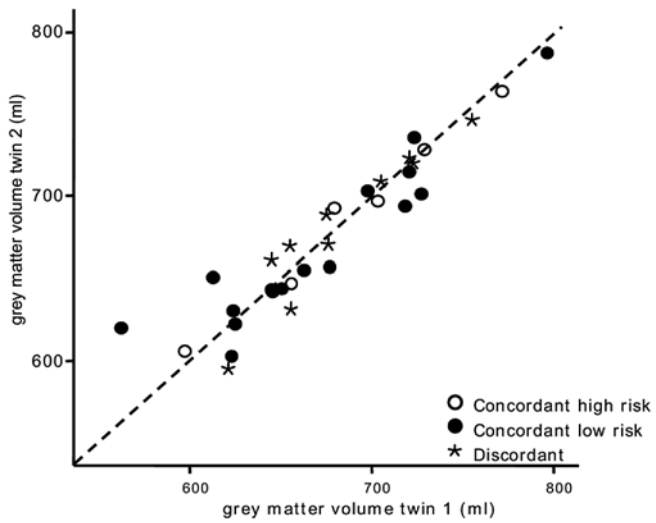

Figure 1: Global gray-matter volumes (in mL) of individual twin pairs. Every symbol corresponds to one pair, with brain size of one twin on the horizontal and brain size of the other twin on the vertical axis. The dashed vertical line represents the identity line (gray-matter volume of twin 1 = gray-matter 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.

close-up view, with voxel *p* value threshold raised to $p<0.01$ (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.

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 $p<0.05$.

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 highcompared 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.

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).

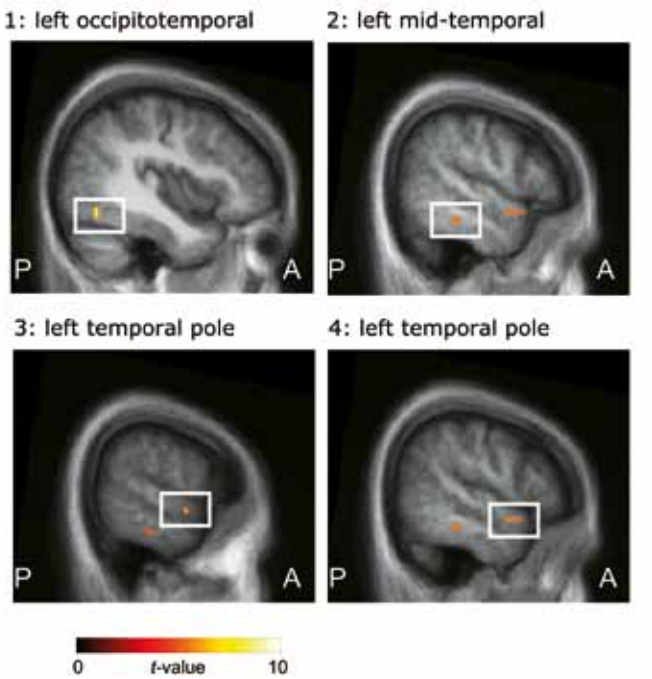

Figure 2: Parametric *t* map of regional gray-matter size reductions in the high-risk, relative to the low-risk, discordant twins. *T* values are mapped by the color bar and projected on the average MR sections of the 20 discordant twins. Four of the five temporal regions that met our criterion of significant volume reduction in the high-risk compared with the low-risk twin ($p < 0.001$; min, 50 voxels) are shown.
A fifth region is shown separately in Figure 3.

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.

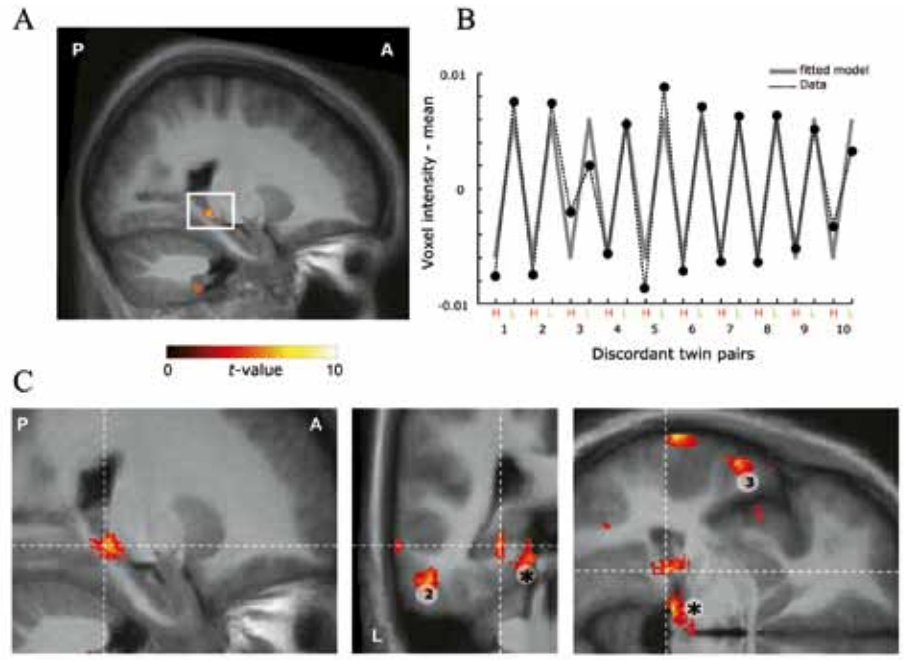

Figure 3: (A) Boxed region contains a left parahippocampal area in which a significant volume reduction was found in the high-risk twin compared within the low-risk twin ($p < 0.001$; min, 50 voxels). (B) Relative responses of the discordant twin pairs at the most significant voxel ($t = 8.08$ at $x = -24$, $y = -34$, $z = -6$ 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 ($p < 0.01$; 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 $x = -9$, $y = -32$, $z = -6$).

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 χ^2, $p< 0.05$).

Discussion

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

References

1. Ashburner J, Neelin P, Collins DL, Evans A, Friston K (1997): Incorporating prior knowledge into image registration. Neuroimage 6:344 –352. Ashburner J, Friston KJ (1999): Nonlinear spatial normalization using basis functions. Hum Brain Mapp 7:254 –266.
2. Ashburner J, Friston KJ (2000): Voxel-based morphometry—The methods. Neuroimage 11:805– 821.
3. Ashburner J, Friston KJ (2001): Why voxel-based morphometry should be used. Neuroimage 14:1238 –1243.
4. Ansorge MS, Zhou M, Lira A, Hen R, Gingrich JA (2004): Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. Science 306:879–881.
5. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961): An inventorymeasuring depression. Arch Gen Psychiatry 4:53– 63.
6. Bell-McGinty S, Butters MA, Meltzer CC, Greer PJ, Reynolds CF 3rd, Becker JT(2002): Brain morphometric abnormalities in geriatric depression: Longtermneurobiological effects of illness duration. Am J Psychiatry 159:1424–1427.
7. Boomsma DI, Beem AL, van den Berg M, Dolan CV, Koopmans JR, Vink JM, *et al.* (2000): Netherlands twin family study of anxious depression (NETSAD). Twin Res 3:323–334.
8. Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, *et al.* (1995): MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 152: 973–981.
9. Campbell S, Marriott M, Nahmias C, MacQueenGM(2004): Lower hippocampal volume in patients suffering from depression: A meta-analysis. Am J Psychiatry 161:598–607.
10. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, *et al.* (2003): Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science 301:386 –389.
11. Castellanos FX, Sharp WS, Gottesman RF, Greenstein DK, Giedd JN, Rapoport JL (2003): Anatomic brain abnormalities in monozygotic twins discordant for attention deficit hyperactivity disorder. Am J Psychiatry 160: 1693–1696.
12. Czeh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, *et al.* (2001): Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci U S A 98:12796 –12801.
13. DumanRS, Malberg J,ThomeJ. Neural plasticity to stress and antidepressant treatment (1999): Biol Psychiatry 46:1181–1191.
14. Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, *et al.* (2004): Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol Psychiatry 9:908 –915.
15. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, *et al.* (2005):Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A 102:10604–10609.
16. Freeman TW, Cardwell D, Karson CN, Komoroski RA (1998): *In vivo* proton magnetic resonance spectroscopy of the medial temporal lobes of subjects with combat-related posttraumatic stress disorder. Magn Reson Med 40:66 –71.
17. Geuze E, Vermetten E, Bremner JD (2005): MR-based *in vivo* hippocampal volumetrics: 1. Review of methodologies currently employed. Mol Psychiatry 10:147–159.
18. Gilbertson MW, Shenton ME, Ciszewski A, Kasai K, Lasko NB, Orr SP, Pitman RK (2002): Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. Nat Neurosci 5:1242–1247.
19. Good CD, Johnsrude IS, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ (2001):Avoxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage 14:21–36.
20. Grabe HJ, Lange M, Wolff B, Volzke H, Lucht M, Freyberger HJ, *et al.* (2005): Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden. Mol Psychiatry 10:220 –224.
21. Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, *et al.* (2002): Serotonin1A receptor acts during development to establish normal anxiety- like behaviour in the adult. Nature 416:396–400.
22. Gurvits TV, Shenton ME, Hokama H, Ohta H, Lasko NB, Gilbertson MW, *et al.* (1996): Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. Biol Psychiatry 40:1091–1099.
23. Hariri AR, Drabant EM, Munoz KE, Kolachana BS, Mattay VS, Egan MF, Weinberger DR (2005): A susceptibility gene for affective disorders and the response of the human amygdala. Arch Gen Psychiatry 62:146 –152.
24. Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, *et al.* (2002): Serotonin transporter genetic variation and the response of the human amygdala. Science 297:400–403.
25. Hariri AR, WeinbergerDR(2003): Imaging genomics. BrMedBull 65:259 –270.
26. Herman JP, Cullinan WE (1997): Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20: 78–84.
27. Hettema JM, Prescott CA, Kendler KS (2001): A population-based twin study of generalized anxiety disorder in men and women. J Nerv Ment Dis 189:413– 420.
28. Hsieh MH, McQuoid DR, Levy RM, Payne ME, MacFall JR, Steffens DC (2002): Hippocampal volume and antidepressant response in geriatric depression. Int J Geriatr Psychiatry 17:519 –525.

29.Hyde TM, Stacey ME, Coppola R, Handel SF, Rickler KC, Weinberger DR (1995): Cerebral morphometric abnormalities in Tourette's syndrome: A quantitative MRI study of monozygotic twins. Neurology 45:1176 –1182.

30.Jacobs BL, Praag H, Gage FH (2000): Adult brain neurogenesis and psychiatry: A novel theory of depression. Mol Psychiatry 5:262–269.

31.Kaufman J, Yang BZ, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal JH, Gelernter J (2004): Social supports and serotonin transporter gene moderate depression in maltreated children. Proc Natl Acad Sci U S A 101:17316–17321.

32.Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B (2005): The interaction ofstressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: A replication. Arch Gen Psychiatry 62:529 –535.

33.endler KS, Myers J, Prescott CA, NealeMC(2001): The genetic epidemiology of irrational fears and phobias in men. Arch Gen Psychiatry 58:257–265.

34.Lavenex P, Amaral DG (2000): Hippocampal-neocortical interaction: A hierarchy of associativity. Hippocampus 10:420–430.

35.Lesch KP, Gutknecht L (2005): Pharmacogenetics of the serotonin transporter. Prog Neuropsychopharmacol Biol Psychiatry 29:1062–1073. Levinson DF (2006): The genetics of depression: A review. Biol Psychiatry 15;60:84 –92.

36.Lopez JF, Chalmers DT, Little KY, Watson SJ (1998): Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: Implications for the neurobiology of depression. Biol Psychiatry 43:547–573.

37.Luteijn F, van der Ploeg FAE (1983): Handleiding Groninger Intelligentie Test (GIT) [Manual for the Groningen Intelligence Test]. Lisse, The Netherlands: Swets and Zeitlinger.

38.Lyons DM, Yang C, Sawyer-Glover AM, Moseley ME, Schatzberg AF (2001): Early life stress and inherited variation in monkey hippocampal volumes. Arch Gen Psychiatry 58:1145–1151.

39.MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, *et al.* (2003): Course of illness, hippocampal function, and hippocampal volume in major depression. Proc Natl Acad Sci U S A 100:1387–1392.

40.McEwen BS (1999): Stress and hippocampal plasticity. Annu Rev Neurosci 22:105–122.

41.McEwen BS (2001): From molecules to mind. Stress, individual differences, and the social environment. Ann N Y Acad Sci 935:42– 49.

42.McEwen BS, Magarinos AM (1997): Stress effects on morphology and function of the hippocampus. Ann N Y Acad Sci 821:271–284.

43.McEwen BS, Magarinos AM (2001): Stress and hippocampal plasticity: Implications for the pathophysiology of affective disorders. Hum Psychopharmacol 16(S1):S7–S19.

44.Middeldorp CM, Cath DC, van den BM, Beem AL, Van Dyck R, Boomsma DI (2006): The association of personality with anxious and depressive psychopathology. In: T Canli, ed. The Biological Basis of Personality and Individual Differences. New York: Guilford Press, 251–272.

45.Montgomery SA, Asberg M (1979): A new depression scale designed to be sensitive to change. Br J Psychiatry 134:382–389.

46.Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, *et al.* (2002): Preclinical models: Status of basic research in depression. Biol Psychiatry 52:503–528.

47.Neumeister A, Wood S, Bonne O, Nugent AC, Luckenbaugh DA, Young T, *et al.* (2005): Reduced hippocampal volume in unmedicated, remitted patients with major depression versus control subjects. Biol Psychiatry 15; 57:935–937.

48.Posener JA, Wang L, Price JL, Gado MH, Province MA, Miller MI, *et al.* (2003): High-dimensional mapping of the hippocampus in depression. Am J Psychiatry 160:83– 89.

49.Roozendaal B (2003): Systems mediating acute glucocorticoid effects on memory consolidation and retrieval. Prog Neuropsychopharmacol Biol Psychiatry 27:1213–1223.

50.SapolskyRM(2000): Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57:925–935.

51.Sapolsky RM, Krey LC, McEwen BS (1986): The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. Endocr Rev 7:284– 301.

52.Sen S, Burmeister M, Ghosh D (2004): Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits.AmJ Med Genet B Neuropsychiatr Genet 127:85– 89.

53.Sheline YI, Gado MH, Kraemer HC (2003): Untreated depression and hippocampal volume loss. Am J Psychiatry 160:1516 –1518.

54.Spielberger CD, Gorsuch RL, Lushene RE (1970): STAI Manual for the State- Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press.

55.Starkman MN, Gebarski SS, Berent S, Schteingart DE (1992): Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biol Psychiatry 32:756 –765.

56.Starkman MN, Giordani B, Gebarski SS, Berent S, Schork MA, Schteingart DE (1999): Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. Biol Psychiatry 46:1595–1602.

57.Sullivan EV, Pfefferbaum A, Swan GE, Carmelli D (2001): Heritability of hippocampal size in elderly twin men: Equivalent influence from genes and environment. Hippocampus 11:754 –762.

58.Sullivan PF, Neale MC, Kendler KS (2000): Genetic epidemiology of major depression: Review and meta-analysis. Am J Psychiatry 157:1552–1562.

59.Uno H, Eisele S, Sakai A, Shelton S, Baker E, DeJesus O, Holden J (1994): Neurotoxicity of glucocorticoids in the primate brain. Horm Behav 28: 336–348.

60.Vakili K, Pillay SS, Lafer B, Fava M, Renshaw PF, Bonello-Cintron CM, Yurgelun- Todd DA (2000): Hippocampal volume in primary unipolar major depression: A magnetic resonance imaging study. Biol Psychiatry 47: 1087–1090.

61.Vermetten E, Vythilingam M, Southwick SM, Charney DS, Bremner JD (2003): Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. Biol Psychiatry 54:693–702.

62.Videbech P, Ravnkilde B (2004): Hippocampal volume and depression: A meta-analysis of MRI studies. Am J Psychiatry 161:1957–1966.

63.Vythilingam M, Heim C, Newport J, Miller AH, Anderson E, Bronen R, *et al.* (2002): Childhood trauma associated with smaller hippocampal volume in women with major depression. Am J Psychiatry 159:2072–2080.

64.Watanabe Y, Gould E, Daniels DC, Cameron H, McEwen BS (1992): Tianeptine attenuates stress-induced morphological changes in the hippocampus. Eur J Pharmacol 222:157–162.

65.WechslerD(1997): WAIS-III Wechsler Adult Intelligence Scale. San Antonio, TX: Psychological Corporation.

66.Wittchen HU (1994): Reliability and validity studies of the WHO Composite International Diagnostic Interview (CIDI): A critical review. J Psychiatr Res 28:57– 84.

67.Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC (1996): A unified statistical approach for determining significant signals in images of cerebral activation. Hum Brain Mapp 4:58 –73.

68.Wright IC, McGuire PK, Poline JB, Travere JM, Murray RM, Frith CD, *et al.* (1995): A voxel-based method for the statistical analysis of grey and white matter density applied to schizophrenia. Neuroimage 2:244 –252.

SECTION C

Molecular Neuroimaging

5

First evaluation of [^{11}C]R116301 as an *in vivo* tracer of NK1 receptors in man

Wolfensberger SP, van Berckel BN, Airaksinen AJ, Maruyama K, Lubberink M, Boellaard R, Carey WD, Reddingius W, Veltman DJ, Windhorst AD, Leysen JE, Lammertsma AA.

Molecular Imaging and Biology. 2009 Jul-Aug;11(4):241-5. Epub 2009 Mar 31

Abstract

Purpose

NK1 receptors have been implicated in various neuropsychiatric and other disorders. R116301 is a selective NK1 receptor antagonist. In this pilot study, [^{11}C]R116301 was evaluated as a potential positron emission tomography (PET) ligand for the NK1 receptor.

Procedures

Two dynamic PET studies were performed in three normal volunteers before and after a blocking dose of aprepitant. Data were analyzed using striatum to cerebellum standardized uptake value (SUV) ratios.

Results

Baseline SUV ratios at 60–90 min after injection ranged from 1.22 to 1.70. Following aprepitant administration, this specific signal was completely blocked. Aprepitant administration did not significantly affect uptake in cerebellum, confirming the absence of NK1 receptors in cerebellum.

Conclusion

These preliminary results indicate that [^{11}C]R116301 has potential as a radioligand for *in vivo* assessment of NK1 receptors in the human brain.

Introduction

Tachykinins are a class of neuropeptides that are involved in a variety of biological functions in the central and peripheral nervous systems[1–3]. Tachykinin receptors have been divided into three subtypes according to their preferred ligands: neurokinin 1 (NK1), NK2, and NK3. NK1 receptors, for which substance P has the highest affinity, have been of particular interest because of their potential implication in various neuro-psychiatric and other disorders. Autoradiography studies of the brain of various species have demonstrated the presence of NK1 receptors in several cerebral structures with the highest density in striatum[4] and negligible density in cerebellum[5]. NK1 antagonists could potentially be used for treating a variety of disorders[6–8]. Originally, selective nonpeptide antagonists of the NK1 receptor were studied as potential analgesic compounds[9]. Several studies have provided evidence that NK1 receptor antagonists might be effective in the treatment of anxiety disorders and depression[10–13], although a recent clinical study in depression could not confirm these findings[14]. The highly selective NK1 antagonist aprepitant[14], 5-[[(2R,3S]-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorphenyl)-4-morpholinyl]methyl]-1,2-dihydro3H-1,2,4-triazol-3-one, however, is effective in the treatment of nausea and emesis after chemotherapy and is already in routine clinical use as an anti-emetic agent[15, 16]. To assess novel drugs targeting the NK1 receptor, development of a positron emission tomography (PET) tracer for this target is warranted, as this would provide a unique *in vivo* means of measuring receptor occupancy. In addition, *in vivo* imaging studies might provide new possibilities for studying the role of the NK1 receptor in neuro-psychiatric disorders.

Although several NK1 agonists and antagonists have been labeled successfully for in vitro use, to date, most attempts to develop tracers for *in vivo* imaging studies have not been successful[17–19]. For example, although the radioiodinated selective NK1 antagonist L-703606 was useful for labeling NK1 receptors *in vitro*[20], it failed to provide a specific signal *in vivo* in rat and monkey[21]. For the high affinity antagonist [^{11}C]GR205171, promising results were obtained in monkeys[22], providing images that reflected specific binding to NK1 receptors. Its use was limited, however, by the fact that it did not reach equilibrium within 90 min, which is close to the maximum scanning time possible with a carbon-11 labeled ligand. Recently, [2-[^{18}F]fluoromethoxy-5-(5-trifluoromethyltetrazol-1-yl)benzyl]([2S,3S]-2-phenylpiperidin-3-yl)-amine ([^{18}F]SPARQ) was developed as a fluorine-18 labeled alternative. SPARQ is structurally related to GR205171 and is a selective high affinity antagonist of the NK1 receptor[4, 21, 23]. Unfortunately, in areas with high receptor densities, [^{18}F]SPARQ only reached equilibrium after 6 h, limiting its use for clinical or intervention studies[5]. In addition, plasma kinetics of [^{18}F]SPARQ were fast, and 90 min after injection, the amount of parent compound in blood was too low to be measured reliably[5]. As such, at present there is no “ideal” tracer

for human NK1 receptors and, therefore, alternative compounds need to be investigated.
N1-(2,6-Dimethylphenyl)-2-(4-{(2R,4S)-2-benzyl-1-[3,5i(trifluoromethyl) benzoyl] hexahydro-4-pyridinyl}piperazino)acetamide (R116301) is an orally active, potent, and selective nonpeptide NK1 receptor antagonist with a K_i of 0.45 nM against human NK1 receptors[24]. The affinity for human NK2 and NK3 receptors is 1600-and 230-fold lower, respectively. At high concentrations, R116301 interacts with rat Na^+ and Ca^{2+} ion channel binding sites (K_i> 2 μM), but it does not interact with other receptors, ion channels, or transporter sites at a concentration of 10 μM. R116301 suppresses various aspects of substance P induced behavior *in vivo* and behaves as a full antagonist *in vitro*[25]. Recently, R116301 was labeled with carbon-11[24]. The present study is the first application of [11C]R11 6301 in humans. The aim of the study was to evaluate presence of selective [11C]R116301 binding to the NK1 receptor *in vivo*.

Materials and methods

Subjects

Three healthy subjects, two men aged 46 and 42 and one woman aged 25, were included. Subjects were studied within 21 days after eligibility screening, which consisted of medical history including Structured Clinical Interview for DSM-IV Axis I disorders[26], history of alcohol and drug abuse, physical examination, vital signs and electrocardiogram, laboratory (blood and urine) assessments, and extensive drugs (of abuse) screening. The female subject was practicing an effective method of birth control, and pregnancy tests were performed at screening and on the day of the study. Volunteers were medication-free for at least 14 days prior to PET scanning. In addition, subjects underwent a structural magnetic resonance imaging (MRI) scan within 14 days prior to the actual PET study to exclude any clinically significant abnormalities and for coregistration with the PET images. The study was approved by the Medical Ethics Committee of the VU University Medical Centre. Subjects gave written informed consent prior to entering the study.

Scanning protocol

Each PET session consisted of two [11C]R116301 scans on the same day, separated by 5 h. [11C]R116301 was synthesized as described previously[24]. The first scan was under baseline conditions, while the second was performed 3.5 h after a single oral dose of 125 mg of the NK1 antagonist aprepitant (EMEND®). Pretreatment with 125 mg aprepitant is expected to nearly block specific uptake of [11C]R116301, as it has been reported that this dose leads to an occupancy of NK1 receptors of more than 90%[4].

PET scans were acquired using an ECAT EXACT HR+ PET scanner (Siemens/CTI, Knoxville, TN, USA)[27]. All subjects received an indwelling radial artery cannula for arterial sampling. After a 10 min 2D transmission scan, used to correct the subsequent emission scan for tissue attenuation, a 3D dynamic [11C]R116301 scan was performed. This scan consisted of 23 frames with progressive increase in frame duration (1 x 15, 3 x 5, 3 x 10, 2 x 30, 3 x 60, 2 x 150, 2 x 300, and 7 x 600 s), resulting in a total scan duration of 90 min. The mean (±SD) [11C]R116301 tracer dose, administered using an injector (Multilevel CT Injector; Medrad, Pittsburgh, PA, USA), was 427±44 MBq, with a specific activity of 31± 7 GBq/μmol.
During the [11C]R116301 scan, arterial blood was monitored continuously using an on-line detection system (Veenstra Insruments, Joure, Netherlands)[28]. In addition, at set times manual blood samples were collected for measurement of metabolite fractions.
Magnetic resonance imaging was performed using a 1.5-T Sonata system (Siemens, Erlangen, Germany) with a standard receiver head coil. For each subject, a coronal 3D gradient-echo T1-weighted sequence (matrix 256 x 160; inversion time, 300 ms; TR=15 ms; TE=7 ms; flip angle=80°; voxel size 1 x 1 x 1.5 mm; 160 sections) was acquired.

Image analysis

[11C]R116301 scans were reconstructed using a FORE + 2D filtered back projection algorithm[29] and a Hanning filter with a cut-off at 0.5 times the Nyquist frequency, including usual corrections for decay, dead time, attenuation, scatter, and randoms. A zoom factor of 2 and an image matrix size of 256 x 256 were used, resulting in a final image resolution of ~7 mm full width at half maximum and a voxel size of 1.2 x 1.2 x 2.4 mm^3.
Baseline and post-aprepitant dynamic PET scans were coregistered to each other and subsequently to individual MRI scans using the software package MIRIT[30, 31]. Using anatomical criteria and the threedimensional software program DISPLAY (http://www.bic.mni.mcgill.ca/ software/Display), regions of interest (ROIs) were defined manually on the individual MRI scan for the following structures: caudate, putamen, medial temporal lobe (MTL), thalamus, cingulate, frontal cortex, parietal cortex, insular cortex, occipital cortex, and cerebellum. MRI scans were segmented into grey and white matter and, except for cerebellum, only grey matter voxels within a ROI were used for further analysis[32]. As segmentation in the cerebellum is less accurate, all voxels within the cerebellum ROI were used. Finally, tissue time–activity curves were generated by projecting these ROIs onto all dynamic frames.

Assessment of specific signal

During the study, significant sticking of [11C]R116301 to the wall of the polytetrafluorethylene tubing was suspected, as flushing the line did not result in a reduction of detector counts.

This was confirmed in a retrospective experiment, where significant residual activity was measured after rinsing a tube that had been filled with [11C]R116301 containing blood. Due to this sticking, arterial input curves were unreliable, making compartmental analysis impossible. Therefore, for this first analysis, standardized uptake values (SUV) were used. SUV represents the mean (over a time interval) tissue concentration, normalized for injected dose and either subject weight, lean body mass, or body surface area[33]. SUV were obtained for all ROIs of both baseline and post-aprepitant [11C]R116301 scans. As it is known that the level of NK1 receptors is negligible in cerebellum[5], the target to cerebellum SUV ratio (SUVr) was used for estimating the size of the specific signal, and this ratio was also used as outcome parameter for assessing the effects of aprepitant. Emphasis was on striatum, as this structure has the greatest density of NK1 receptors.

Results

Plasma clearance of [11C]R116301 was rapid with no differences between pre-and post-aprepitant scans (data not shown). Due to this rapid plasma clearance, metabolites could not be measured after 60 min. Metabolite fractions (determined using the manual samples) at 40 min post injection ranged from 20% to 45% (average 35%).

As expected, highest uptake was observed in striatum, with intermediate uptake in cortical areas, and lowest uptake in white matter and cerebellum. In Fig. 1a, pre-and postaprepitant SUV images (60–90 min post injection) are shown for one of the subjects (subject 1), illustrating substantial reduction of [11C]R116301 uptake after aprepitant administration. In contrast to striatum, aprepitant administration did not significantly affect [11C]R116301 SUV in cerebellum, confirming absence of NK1 receptors in cerebellum (Fig. 1b).

Striatum to cerebellum SUVr steadily increased over time but tended to level off after about 60 min post injection (Fig. 1c). Therefore, for assessment of the size of the specific signal, the interval 60–90 min post injection was used.

Baseline striatum to cerebellum SUVr were 1.56, 1.70, and 1.22 and reduced to 0.97, 0.96, and 1.01 post-aprepitant, respectively. Pre-and post-aprepitant SUVr for all ROIs are shown in Table 1, together with their ratio. Note that, in subject number 3, the latter ratio is smaller than 1 for parietal cortex and thalamus, which is probably within measurement error, at least for parietal cortex, although a small movement artifact for thalamus cannot be excluded. More importantly, pre-to post-aprepitant ratios of SUVr ranged from 1.21 to 1.77 for striatum (Table 1).

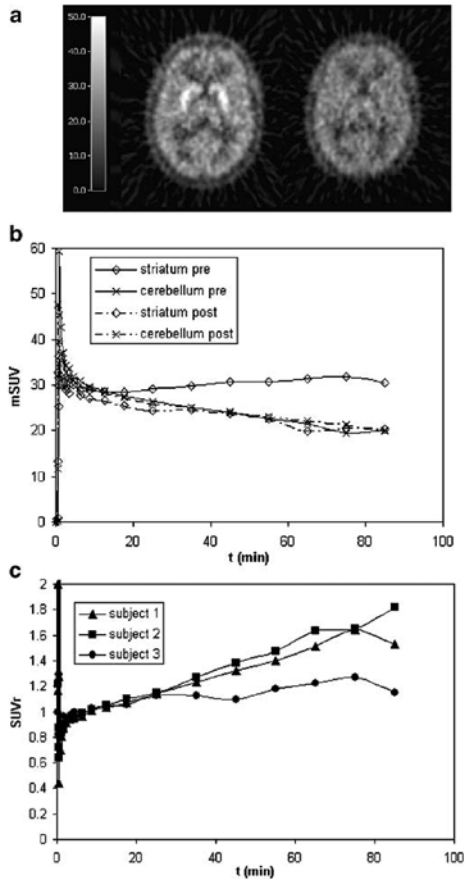

Figure 1: **a** [11C]R116301 SUV images (60–90 min post injection) for subject number 1, before *(left)* and 3.5 h after *(right)* oral administration of 125 mg aprepitant. **b** Pre- and post-aprepitant SUV curves for striatum and cerebellum of subject 1. **c** Pre-aprepitant striatum to cerebellum SUVr. Note that SUV is normalized to body surface area and that results are expressed as milliSUV (mSUV).

Table 1. Pre- and post-aprepitant SUVr (60–90 min post injection), together with their ratio

	Subject 1			Subject 2			Subject 3		
	Pre	Post	Pre/post	Pre	Post	Pre/post	Pre	Post	Pre/post
Striatum	1.56	0.97	1.61	1.70	0.96	1.77	1.22	1.01	1.21
Caudate	1.33	0.85	1.57	1.55	0.86	1.80	1.04	0.92	1.13
Putamen	1.95	1.19	1.64	1.95	1.16	1.69	1.61	1.19	1.35
Thalamus	1.14	0.98	1.16	1.30	1.12	1.16	0.83	102	0.81
Frontal Ctx	1.22	1.00	1.22	1.24	1.02	1.22	0.97	0.97	1.00
Cingulate	1.27	1.07	1.19	1.22	1.02	1.20	1.12	1.11	1.00
MTL	1.02	0.91	1.12	1.16	0.97	1.20	1.08	1.02	1.06
Insula	1.25	1.07	1.17	1.18	1.05	1.13	1.11	1.07	1.04
Parietal Ctx	1.24	1.00	1.24	1.24	0.99	1.24	0.93	0.99	0.95
Occipital Ctx	1.29	1.06	1.22	1.29	1.01	1.27	1.14	1.08	1.05

Ctx cortex, *MTL* medial temporal lobe

Discussion

This is the first study using the highly selective novel PET ligand [11C]R116301 in human subjects. The distribution of this tracer corresponds to the known distribution of NK1 receptors, with high retention in striatum (caudate and putamen) and much lower retention in cortical areas[5]. Blocking studies with the NK1 antagonist aprepitant caused a substantial reduction of the specific signal of [11C]R116301 to background levels, confirming selectivity of binding to the NK1 receptor. On the other hand, the rather low target to cerebellum SUVr suggests a relatively high degree of nonspecific binding in humans.

The signal in the cerebellum could not be blocked by aprepitant, confirming that the density of NK1 receptors in human cerebellum is negligible[5]. Consequently, cerebellum can be used as reference region, i.e., as an estimate of free and nonspecific binding. Therefore, for the blocking studies, the ratio of target to cerebellum SUVr relative to baseline was used as outcome measure. The size of the specific signal was assessed at the interval 60–90 min post injection, as SUVr seemed to become fairly constant within this time span.

It should be noted that striatum to cerebellum SUVr for the baseline scan of subject 3 was lower than that of the other two subjects, indicating lower specific binding (Fig. 1c). Subject 3 was, however, screened in the same manner as the other two subjects, and all parameters were within normal range. Although a moderate age effect (7% decrease per decade, significant in other regions than striatum) on NK1 receptor density has been reported[34], both age and gender could not explain this lower binding, as subject 3 was a man of 42 years. With the present sample size, it is not possible to assess whether the observed intersubject variability was due to biology (e.g., polymorphism with modulating effects on affinity of the receptor) or methodology (i.e., effect of using a simple SUVr for quantification). Further studies are needed to address these issues, in particular assessing the validity of using SUVr as outcome measure. In addition, a tracer kinetic model for more quantitative analyses of [11C]R116301 data needs to be developed.

At present, [18F]SPARQ is a PET tracer that provides a much higher specific signal than [11C]R116301. Therefore, at least in theory, it should provide a more accurate assessment of NK1 receptor status or occupancy. Nevertheless, for clinical applications, [18F]SPARQ is far from ideal because of its slow kinetics, requiring very long study durations, thereby increasing the risk of movement artifacts. At the cost of a reduced signal, [11C]R116301 has the advantage that studies can be performed within a reasonable time (90 min).

When ranking the affinity of various NK1 ligands based on pK_i values versus [3H]SP (substance P), the following order (from high to low) is obtained: GR205171 > aprepitant > R116301 > substance P[35]. It is likely that the affinity of SPARQ[5] is higher or equal than that of GR205171. It is clear that the affinity of R116301 is lower than that of SPARQ, which is in agreement with the higher specific signal observed with [18F]SPARQ. On the other hand, the lower affinity of R116301 implies that [11C]R116301 could be more sensitive to differences in endogenous substance P concentrations. This would be very interesting in intervention studies, where release of the endogenous ligand could be manipulated by a pharmacological challenge or, for example, a fear stimulus. Clearly, further studies are needed to establish whether this is feasible.

The NK1 receptor has shown to be an enigmatic target for neuroscience drug discovery and development. To date, antagonists for the NK1 receptor have shown efficacy in the prevention of chemotherapy-induced nausea and vomiting. Although both NK1 receptors and substance P are widely distributed in the brain and may be involved in many neuropsychiatric disorders, many clinical trials on anxiety and depression have found no therapeutic effects of antagonist drugs. Nevertheless, Casopitant® (GlaxoSmith Kline), an NK1 antagonist, is still under clinical investigation for a variety of disorders, including phase II studies in anxiety and depression. Availability of a PET tracer could facilitate further clinical exploration of NK1 receptor involvement in a variety of neuropsychiatric disorders and serve as a tool for the development of drugs that target the NK1 receptor. The present study demonstrates that [11C]R116301 could be a potential candidate tracer for those investigations.

References

1. Otsuka M, Yoshioka K (1993) Neurotransmitter functions of mammalian tachykinins. Physiol Rev 73:229–308
2. Quartara L, Maggi CA (1997) The tachykinin NK1 receptor. Part I: ligands and mechanisms of cellular activation. Neuropeptides 31:537–563
3. Quartara L, Maggi CA (1998) The tachykinin NK1 receptor. Part II: distribution and pathophysiological. Neuropeptides 32:1–49
4. Hargreaves R (2002) Imaging substance P receptors (NK1) in the living human brain using positron emission tomography. J Clin Psychiatry 63 (Suppl 11):18–24
5. Hietala J, Nyman MJ, Eskola I *et al* (2005) Visualization and quantification of neurokinin-1 (NK1) receptors in the human brain. Mol Imag Biol 7:262–272
6. Farthing MJG (2004) Novel agents for the control of secretory diarrhoea. Expert Opin Investig Drugs Expert 13:777–785
7. Hosoki R, Yanagisawa M, Onishi Y, Yoshioka K, Otsuka M (1998) Pharmacological profiles of new orally active nonpeptide tachykinin NK1 receptor antagonists. Eur J Pharmacol 341:235–241
8. Walpole CSJ, Brown MCS, James IF *et al* (1998) Comparative, general pharmacology of SDZ NKT 343, a novel, selective NK1 receptor antagonist. Br J Pharmacol 124:83–92
9. De Felipe C, Herrero JF, O'Brien JA *et al* (1998) Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature 392:394–397
10. Kent JM, Mathew SJ, Gorman JM (2002) Molecular targets in the treatment of anxiety. Biol Psychiatry 52:1008–1030
11. Kramer MS, Cutler N, Feighner J *et al* (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281:1640–1645
12. Krishnan KRR (2002) Clinical experience with substance P receptor (NK1) antagonists in depression. J Clin Psychiatry 63:25–29
13. Rupniak NM (2002) Elucidating the antidepressant actions of substance P (NK1 receptor) antagonists. Curr Opin Investig Drugs 3:257–261
14. Keller M, Montgomery S, Ball W *et al* (2006) Lack of efficacy of the substance P (neurokinin(1) receptor) antagonist aprepitant in the treatment of major depressive disorder. Biol Psychiatry 59:216–223
15. Dando TM, Perry CM (2004) Aprepitant-A review of its use in the prevention of chemotherapy-induced nausea and vomiting. Drugs 64:777–794
16. Hesketh PJ, Grunberg SM, Gralla RJ *et al* (2003) The oral neurokinin-1 antagonist aprepitant for the prevention of chemotherapy-induced nausea and vomiting: a multinational, randomized, double-blind, placebo-controlled trial in patients receiving high-dose cisplatin-The Aprepitant Protocol 052 Study Group. J Clin Oncol 21:4112–4119

17. Breeman WAP, VanHagen MP, VisserWisselaar HA *et al* (1996) In vitro and *in vivo* studies of substance P receptor expression in rats with the new analog [indium-111-DTPA-Arg(1)] substance P. J Nucl Med 37:108–117
18. Delrosario RB, Mangner TJ, Gildersleeve DL *et al* (1993) Synthesis of a nonpeptide C-11 labeled substance-P antagonist for PET studies. Nucl Med Biol 20:545–547
19. Livni E, Babich JW, Desai MC *et al* (1995) Synthesis of a C-11 labeled Nk1 receptor-ligand for PET studies. Nucl Med Biol 22:31–36
20. Francis BE, Swain C, Sabin V, Burns HD (1994) Radioiodinated L703,606—a potent, selective antagonist to the human NK(1) receptor. Appl Radiat Isotopes 45:97–103
21. Solin O, Eskola O, Hamill TG *et al* (2004) Synthesis and characterization of a potent, selective, radiolabeled substance-P antagonist for NK1 receptor quantitation: ([F-18]SPA-RQ). Mol Imag Biol 6:373–384
22. Bergstrom M, Fasth KJ, Kilpatrick G *et al* (2000) Brain uptake and receptor binding of two [C-11]labelled selective high affinity NK1 antagonists, GR203040 and GR205171—PET studies in rhesus monkey. Neuropharmacology 39:664–670
23. Bergstrom M, Hargreaves RJ, Burns HD *et al* (2004) Human positron emission tomography studies of brain neurokinin 1 receptor occupancy by aprepitant. Biol Psychiatry 55:1007–1012
24. Van der Mey M, Janssen CGM, Janssens FE *et al* (2005) Synthesis and biodistribution of [C-11]R116301, a promising PET ligand for central NK1 receptors. Bioorg Med Chem 13:1579–1586
25. Megens AA, Ashton D, Vermeire JC *et al* (2002) Pharmacological profile of (2R-trans)-4-[1-[3,5-bis(trifluoromethyl)benzoyl]-2-(phenylmethyl)-4-piper idinyl]-N-(2,6-dimethylphenyl)-1-acetamide (S)hydroxybutanedioate (R116301), an orally and centrally active neurokinin-1 receptor antagonist. J Pharmacol Exp Ther 302:696–709
26. First MB, Spitzer RL, Gibbon M, Williams JBW (1996) Structured clinical interview for DSM-IV clinical version (SCID-I/CV). American Psychiatric Press, Washington, DC
27. Brix G, Zaers J, Adam LE *et al* (1997) Performance evaluation of a whole-body PET scanner using the NEMA protocol. J Nucl Med 38:1614–1623
28. Boellaard R, van Lingen A, van Balen SCM, Hoving BG, Lammertsma AA (2001) Characteristics of a new fully programmable blood sampling device for monitoring blood radioactivity during PET. Eur J Nucl Med 28:81–89
29. Defrise M, Kinahan PE, Townsend DW, Michel C, Sibomana M, Newport DF (1997) Exact and approximate rebinning algorithms for 3D PET data. IEEE Trans Med Imag 16:145–158
30. Maes F, Collignon A, Vandermeulen D, Marchal G, Suetens P (1997) Multimodality image registration by maximization of mutual information. IEEE Trans Med Imag 16:187–198

31.West J, Fitzpatrick JM, Wang MY *et al* (1997) Comparison and evaluation of retrospective intermodality brain image registration techniques. J Comput Assist Tomogr 21:554–566
32.Zhang YY, Brady M, Smith S (2001) Segmentation of brain MR images through a hidden Markov random field model and the expectationmaximization algorithm. IEEE Trans Med Imag 20:45–57
33.Hoekstra CJ, Paglianiti I, Hoekstra OS *et al* (2000) Monitoring response to therapy in cancer using [F-18]-2-fluouo-2-deoxy-D-glucose and positron emission tomography: an overview of different analytical methods. Eur J Nucl Med 27:731–743
34.Nyman MJ, Eskola O, Kajander J *et al* (2007) Gender and age affect NK1 receptors in the human brain-a positron emission tomography study with [F-18]SPA-RQ. J Comput Assist Tomogr 10:219–229
35.Griffante C, Carletti R, Andreetta F, Corsi M (2006) [3H]GR205171 displays similar NK1 receptor binding profile in gerbil and human brain. Br J Pharmacol 148:39–45

6

Quantification of the NK1 Receptor ligand [^{11}C]R116301

Wolfensberger SP, Maruyama K, van Berckel BN, Lubberink M, Airaksinen AJ, Boellaard R, Luurtsema G, Reddingius W, Janssens FE, Veltman DJ, Windhorst AD, Leysen JE, Lammertsma AA

Submitted

Abstract

Purpose

NK1 receptors are potential targets for therapeutic agents, as they have been implicated in depression, anxiety and pain perception. Recently, it was shown that, in human brain, a specific NK1 receptor related signal was obtained with the novel radioligand [^{11}C]R116301 using positron emission tomography (PET). The purpose of the present study was to evaluate various methods for quantifying specific [^{11}C]R116301 binding.

Methods

Two dynamic 90 minutes [^{11}C]R116301 scans, separated by 5 hours, were performed in 11 healthy volunteers. In three subjects, the second scan was performed after an oral blocking dose of 125 mg aprepitant, whilst in the other 8 no intervention was performed (test-retest). Whole striatum was used as tissue of interest, as it has the highest density of NK1 receptors. Cerebellum was used as reference tissue.

Results

No useful results were obtained with plasma input models due to extensive sticking of the tracer to the tubing of the blood sampling device. Measured plasma curves were not reliable even after implementing a validated sticking correction. Reference tissue models were stable with the simplified reference tissue model (SRTM) performing best. Average (± SD) SRTM derived mean binding potential BP_{ND} of all (first) baseline scans was 0.64 ± 0.31 (n = 11), which reduced to –0.01 ± 0.03 (n = 3) following aprepitant administration. Test-retest results showed low variability (14.0 ± 10.7%) and excellent reliability, as indicated by the intraclass correlation coefficient (ICC = 0.93). The ratio of standardized uptake values of striatum and cerebellum minus 1 (SUVr-1), an approximation of BP_{ND}, showed very low variability (6.2 ± 3.1%) with excellent reliability (ICC = 0.98), and correlated well with SRTM derived BP_{ND} (R^2 = 0.96).

Conclusions

In the absence of a reliable plasma input function, SRTM is the model of choice for quantifying [^{11}C]R116301 binding. Semi-quantitative tissue ratios hold promise for routine clinical applications.

Introduction

Substance P (SP) is one of the most abundant neuropeptides in the central nervous system. It has been implicated in a variety of neuro-psychiatric processes, including stress-regulation as well as affective and anxiety-related behaviour[1]. SP and its preferred G-protein-coupled neurokinin 1 (NK1) receptor have been found in brain areas known to be involved in the regulation of stress and anxiety responses with the highest density in striatum[2] and negligible density in cerebellum[3]. In these regions, SP frequently coexists in the same neuron with other neurokinins and with 'classical' neurotransmitters such as dopamine, acetylcholine, serotonin, noradrenalin, GABA and glutamate[1].

Over the last decades, NK1 receptors gained interest as a pharmacological target and antagonists for this receptor were thought to be useful for the treatment of depression, anxiety and inflammatory pain[4-6]. In addition, preclinical evidence suggested a role of SP-NK1 in nociception, neurogenesis and emetogenesis[7-9]. The NK1 antagonist, aprepitant (EMEND®) is on the market for treatment of radio- and chemo-therapy induced emesis. Several NK1 antagonists went into advanced clinical trials for treatment of depression (e.g. L759274, phase III; vesipitant, phase II), or anxiety (R673, phase II), but these studies failed. Recently, compounds like casopitant and AV608 are in phase II clinical trials for various indications such as myalgia, insomnia, irritable bowel syndrome and urinary incontinence. PET could be a useful tool to visualise and quantify specific receptor binding in order to gain more insight in neurotransmitter-receptor function and it could aid in assessing the optimal dose range of new drugs for central NK1 receptor occupancy.

N1-(2,6-Dimethylphenyl)-2-(4-{(2R,4S)-2-benzyl-1-[3,5i(trifluoromethyl) benzoyl] hexahydro-4-pyridinyl}piperazino)acetamide (R116301) is an orally active, potent and selective non-peptide NK1 receptor antagonist with a K_i value of 0.45 nM for human NK1 receptors[10]. Recently, it was labelled with carbon-11[10] and in a pilot study in healthy human subjects an NK1 related specific signal could be observed[11].

The purpose of the present study was to evaluate various tracer kinetic models for quantification of [^{11}C]R116301 binding to the NK1 receptor using both blocking and test-retest data.

Methods

Subjects

In total 11 healthy subjects were included in this study. Three healthy subjects, two males aged 46 and 42 and one female aged 25, have previously been included in a pilot study to assess whether a specific signal could be detected *in vivo* by comparing [11C]R116301 scans before and after aprepitant predosing[11] . In the present study these data were used for modelling purposes. Test-retest data were obtained in 8 healthy males aged 19 to 27 years. All subjects were studied after eligibility screening, which consisted of medical history including Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I)[12] and history of alcohol and drug abuse, physical examination, vital signs and ECG, laboratory (blood and urine) assessments, and extensive toxicology screening. Subjects were medication free for at least 14 days prior to the PET study. All subjects underwent a structural MRI scan within 14 days prior to the actual PET study. None of the MRI scans showed any clinical significant abnormalities, as reported by a neuroradiologist. The study was approved by the Medical Ethics Review Committee of the VU University Medical Centre. All subjects gave written informed consent prior to entering the study.

Synthesis of [11C]R116301

GMP compliant production of [11C]R116301 was performed in a government licensed GMP hotlab facility. [11C]R116301 was synthesized according to a previously published method with minor modifications[10] . In short, at a temperature of -65°C, 3,5-bis(trifluoromethyl) bromobenzene was treated with n-butyllithium and [11C]CO_2 was passed through this solution. The reaction mixture was quenched with 1M HCl in diethyl ether to yield [11C]3,5-bis(trifluoromethyl)benzoic acid quantitatively. This product was subsequently transformed to [11C] 3,5-bis(trifluoromethyl)benzoyl chloride (**1**) by treatment with 2M oxalyl chloride in dichloromethane. After removal of excess of oxalyl chloride by evaporation with a stream of Helium at 68°C, the mixture was cooled to -20 °C and a solution of the precursor R187662, *N*1-(2,6-dimethylphenyl)-2-{4-[(2*R*,4*S*)-2-benzylhexahydro-4-pyridinyl] piperazino}acetamide (**2**), in dimethylformamide (DMF) and triethylamine (TEA) was added. The product [11C]R116301 was purified with semipreparative HPLC and isolated by solid phase extraction. As a result, [11C]R116301 was obtained in an average decay-corrected radiochemical yield of 24 ± 6 % (from [11C]CO_2) in a total synthesis time of 35 minutes with a chemical and radiochemical purity exceeding 98% and a specific activity of 28.8 ± 8.6 GBq/µmol at time of injection. The product was formulated in a sterile and pyrogen free mixture of ethanol (1.3 mL), 7.09 mM NaH_2PO_4 in 0.9% NaCl (1.5 mL) and 0.9% NaCl (12 mL).

Five percent human serum albumin (HSA) was added to the formulation to prevent adhesion to the injection system.

Scanning protocol

Each PET session consisted of two [11C]R116301 scans on the same day, separated by 5 hours. In the blocking study, the first scan was under baseline conditions, whilst the second was performed 3.5 hours after a single oral dose of 125 mg of the NK1 antagonist aprepitant (EMEND®). Pre-treatment with 125 mg aprepitant is expected to nearly block specific uptake of [11C]R116301, as it has been reported that this dose leads to an occupancy of NK1 receptors of more than 90%[2]. For the test- retest study exactly the same protocol was used, but without intervention.

PET scans were acquired using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA), which has been described previously[13]. All subjects received an indwelling radial artery cannula for arterial sampling. After a 10 minutes 2D transmission scan, which was used to correct the subsequent emission scan for attenuation, a 3D dynamic emission scan was performed following an intravenous bolus injection of [11C]R116301. This scan consisted of 23 frames with progressive increase in frame length (1x15, 3x5, 3x10, 2x30, 3x60, 2x150, 2x300 and 7x600 s) and a total duration of 90 minutes. Arterial blood was monitored continuously using an online detection system (Veenstra Instruments, Joure, Netherlands)[14] with a withdrawal rate of 5 mL/min during the first 10 minutes and 2.5 mL/min thereafter. PTFE tubing was used to minimise potential sticking of activity to the tube wall. In addition, at set times (5, 10, 20, 40, 60, 75 and 90 minutes post-injection), discrete manual samples were taken. After each sample, the line was flushed with heparinised saline.

Magnetic resonance imaging was performed on a 1.5-T Sonata MR system (Siemens, Erlangen, Germany) with a standard receiver head coil. Anatomic imaging included a coronal 3D gradient-echo T1-weighted sequence (matrix 256 x 160, inversion time: 300 ms, TR = 15 ms; TE = 7 ms; flip angle = 80°; voxel size 1 x 1 x 1.5 mm, 160 sections).

Image analysis

[11C]R116301 scans were reconstructed using a FORE+2D filtered back projection algorithm[15] and a Hanning filter with a cut-off at 0.5 times the Nyquist frequency, resulting in a final image resolution of ~7 mm full width at half maximum. A zoom factor of 2 and an image matrix size of 256x256, were used, resulting in a voxel size of 1.2 x 1.2 x 2.4 mm^3. (All data were normalised and corrected for attenuation, dead time, decay, scatter and randoms.)

The first and second dynamic PET scans were co-registered to each other, and subsequently to the individual MRI scan using the software package MIRIT[16, 17] .

Using anatomical criteria and the three-dimensional software program DISPLAY (http://www.bic.mni.mcgill.ca/software/Display), striatum, consisting of caudate and putamen, and cerebellum were defined manually. Both assessment of blocking effect of aprepitant and test-retest variability was based on striatum, as this structure has the highest density of NK1 receptors. As it is known that the level of NK1 receptors in cerebellum is negligible [3,11,18,19], this structure was used as reference region. MRI scans were segmented into grey and white matter and, except for cerebellum, only grey matter voxels within an ROI were used for further analysis[20]. Tissue time-activity curves (TACs) were generated by projecting these ROIs onto all dynamic frames of both scans.

Kinetic analysis

Striatal TACs were analysed using several analytical methods: plasma input models, reference tissue models and analyses based on standardized uptake values (SUV).

Plasma input models

For plasma input models[21], an accurate metabolite corrected input function is required. Previously, it was shown that plasma input curves were not reliable due to stickiness of [11C]R116301 to the walls of the sampler tubing[11]. Therefore, in the present analysis, a correction for sticking activity, previously validated for [11C]deprenyl, was incorporated[22]. This correction assumes that sticking can be described by a single exponential function. In addition, in the present analysis, also a double exponential approach was implemented to correct for sticking.

Reference Tissue Models

Using cerebellum as indirect input function, both full (FRTM; 4 parameters)[23] and simplified (SRTM; 3 parameters)[24] reference tissue models were evaluated, yielding non-displaceable binding potential BP_{ND}[25] of striatum. In order to stabilize fits, the following boundaries were used: $k_2 > 0.025$, $0.0000001 < R_1 < 2$, and $BP > -0.499$. Although binding cannot be negative, the lower limit of BP_{ND} was set to a negative value to avoid noise induced positive bias in case of negligible binding (as expected in the blocking study). Akaike[26] and Schwarz[27] criteria were used to assess which model best described the data.

Single scan method

Standardized uptake values (SUV) for striatum and cerebellum, together with their ratio (SUVr) were calculated for the interval 60-90 minutes after injection. SUVr–1 was used as an index of binding potential.

Test-retest reproducibility and blocking analysis

Test-retest reproducibility of BP_{ND} and SUVr–1 was assessed in terms of variability and reliability[28]. The within subject variability was defined as the absolute value of the difference between test and retest values, expressed as the percentage of the mean of test and retest values:

$$\text{Variability (\%)} = 100 \times |\text{test} - \text{retest}| \,/\, ((\text{test} + \text{retest})/2)$$

Here test and retest refer to the estimated values of the specific parameter of interest for scans 1 and 2, respectively. VAR < 10% was considered as very low, between 10% and 15% as low, between 15 and 20% as moderate, and > 20% as high. As a measure of reliability the intraclass correlation coefficient (ICC) was used. This is defined by:

$$ICC = (MSBS - MSWS) \,/\, (MSBS + MSWS)$$

where MSBS is the mean sum of squares between subjects, and MSWS is the mean sum of squares within subjects. This coefficient is an estimate of the reliability of the two sets of measurements and ranges from –1 (no reliability) to + 1 (perfect reliability, i.e., identical test and retest measurements). Values close to 1 indicate that most variance is due to between subject rather than within subject variation (good reliability), whereas values below zero imply greater within subject than between subject variation (poor reliability). ICC over 0.90 was considered as excellent, ICC between 0.70 and 0.90 as good and ICC under 0.70 as poor[29].

In addition, occupancy after blocking, as assessed using BP_{ND} and SUVr–1, was calculated according to the following equation:

$$\text{Occupancy (\%)} = 100 \times (1 - \text{scan2}/\text{scan1})$$

where scan1 and scan2 represent observed outcome measures of baseline and 'blocked' PET studies, respectively.

Finally, SUVr–1 was compared with BP_{ND} derived from SRTM to assess the feasibility of using SUVr–1.

Results

Average administered tracer dose across all 22 scans was 390 ± 47 MBq with an average specific activity of 28.8 ± 8.6 GBq/µmol. Average total administered R116301 (cold and hot) was 14.7 ± 4.5 nmol (N=22). There were no statistically significant differences in any of these parameters between test and retest scans, nor between baseline and blocking scans.

Plasma input models

Significant sticking of [^{11}C]R116301 to the wall of the PTFE tubing was observed, as counts detected by the online sampler remained high when flushing the line after taking a manual blood sample. This was confirmed in a separate experiment, where significant residual activity was measured after rinsing a tube filled with blood that contained [^{11}C]R116301.

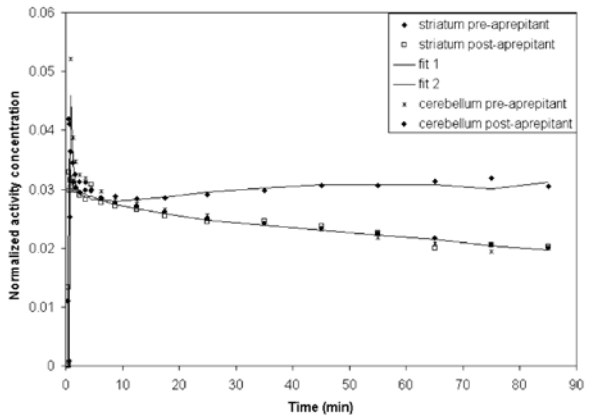

Figure 1: Normalized pre- and post-aprepitant time activity curves for striatum and cerebellum. Lines represent SRTM fits to the striatum data using cerebellum as reference tissue input function.

In an attempt to generate reliable plasma curves, a single exponential sticking correction algorithm, previously validated for [^{11}C]deprenyl[22], was incorporated to characterise binding of [^{11}C]R116301 to the tubing wall. Unfortunately, the shape of the tail of a "corrected" curve did not correspond with that of the corresponding samples. Clearly, a single exponential was not sufficient to characterise sticking and, therefore, a double exponential was attempted. In most cases, however, sticking fits did not converge, indicating that too many parameters were involved for reliable estimation. Consequently, despite attempts to correct for sticking activity, plasma curves remained unreliable, prohibiting the use of plasma input models.

Table 1: Occupancy (%) of NK1 receptors in striatum following predosing with 125 mg aprepitant

Subject No	BP_{ND}	SUVr-1
1	101.9	105.4
2	105.0	105.7
3	87.0	95.5

BP_{ND} based on SRTM; SUVr: ratio of striatum over cerebellum SUV, 60-90 minutes p.i.

Table 2: SRTM derived BP_{ND} for striatum and SUVr-1 for both scans. Averages (±SD) for all baseline scans (scan 1; N=11) are given in the last row

Subject No	BP_{ND} Scan 1	BP_{ND} Scan 2	SUVr-1 Scan 1	SUVr-1 Scan 2
1	0.73	-0.01	0.56	-0.03
2	0.86	-0.04	0.70	-0.04
3	0.15	0.02	0.22	0.01
4	0.44	0.57	0.45	0.46
5	0.99	0.84	0.83	0.75
6	0.05	-0.03	0.16	0.13
7	0.81	0.79	0.65	0.62
8	0.82	0.62	0.66	0.59
9	0.51	0.60	0.52	0.56
10	0.88	0.88	0.71	0.63
11	0.75	0.69	0.64	0.68
Average	0.64±0.31		0.55±0.21	

SUVr: ratio of striatum over cerebellum SUV, 60-90 minutes p.i.
Scan 2: for subjects 1-3 after predosing with aprepitant, for subjects 4-11 retest scan.

Table 3: Test-retest variability (%) of BP_{ND} in striatum and SUVr-1 for all subjects. Averages (±SD) across all subjects are given in the last row

Subject No	BP_{ND}	SUVr-1
4	26.9	4.4
5	16.6	10.1
7	2.7	4.7
8	27.0	9.5
9	16.0	7.4
10	0.5	1.4
11	8.3	6.1
Average	14.0±10.7	6.2±3.1

BP_{ND} based on SRTM; SUVr: ratio of striatum over cerebellum SUV, 60-90 minutes p.i.

Reference tissue models

Representative baseline and post-aprepitant [11C]R116301 TACs for striatum, together with best fits according to SRTM, with corresponding cerebellar TACs are shown in figure 1. After aprepitant administration, striatal TACs were similar to cerebellar TACs, indicating complete occupancy of the NK1 receptor.

According to both Akaike and Schwarz criteria, SRTM was preferred in 21 out of the total of 22 cases. Only in one post-aprepitant scan (subject 1) was a better fit obtained with FRTM. In addition, in case of FRTM, in 18 out of 22 cases standard errors of fitted BP_{ND} were >100% and in another 2 they were >25% (data not shown). Therefore, only BP_{ND} results obtained with SRTM were used for further analysis.

Post-aprepitant BP_{ND} values were close to zero, indicating complete occupancy independent of (variation in) baseline values (Table 1). Mean baseline BP_{ND} (± SD) over all subjects (all baseline, test and retest values; n=19) was 0.63 ± 0.29. Mean baseline BP_{ND} over all subjects (without retest values; n=11) was 0.64 ± 0.31 (Table 2). For one of the subjects (subject number 6) both test and retest BP_{ND} values were close to zero. Although there was a reasonable large range in BP_{ND} values between subjects, only in this subject specific binding appeared to be negligible. As calculations for (relative) test-retest variability are based on absolute differences, and for very low binding levels even minor absolute differences can result in large relative differences, this analysis was applied over all subjects except subject number 6. This resulted in low mean variability (14.0%) (Table 3). Test-retest reliability was excellent (ICC = 0.93).

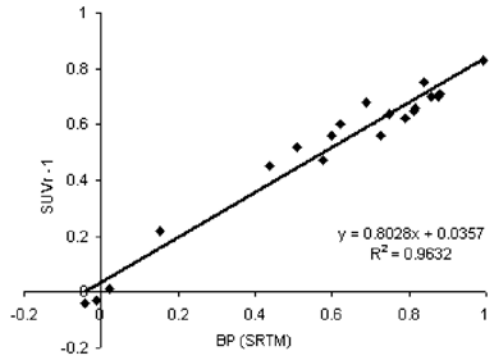

Figure 2: Correlation of SUVr-1 (60-90 min p.i.) with SRTM derived BP_{ND}.

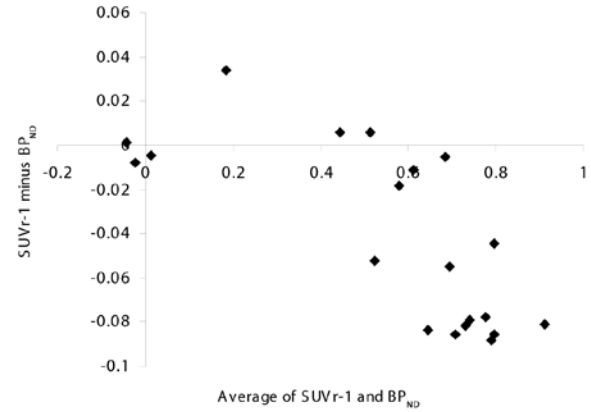

Figure 3: Bland-Altman of SUVr-1 (60-90 min) and SRTM derived BP_{ND}, showing the differences versus the averages. The difference was calculated using SUVr-1 minus BP_{ND}(STRM)

Single scan method

Mean SUVr–1 of baseline scans was 0.55 ± 0.21 (Table 2). As shown in Table 1, aprepitant administration completely blocked the signal, indicating that SUVr–1 indeed reflects specific binding. Test-retest variability (Table 3) was very low (6.2%) and reliability was also excellent (ICC = 0.98).

BP_{ND} vs SUVr-1

To assess the accuracy of SUVr–1, results from both blocking and test-retest studies were compared with those of SRTM derived BP_{ND}. The correlation between both parameters is shown in figure 2. Both outcome measures were highly correlated (R^2 = 0.96), but there was a slight (systematic) underestimation of SUVr–1 as compared with BP_{ND}. A Bland-Altman plot of BP_{ND} obtained with SRTM and SUVr-1 is given in figure 3 showing that SUVr–1 underestimates specific binding at higher BP_{ND} consistent with the slope = ~0.8 in figure 2.

Discussion

The purpose of the present study was to evaluate various different methods for quantification of binding of the novel PET ligand [11C]R116301[10] to NK1 receptors in humans. Recently, in the first human application of [11C]R116301, a specific NK1 related signal was demonstrated using predosing with aprepitant and simple striatum (highest density of NK1 receptors) to cerebellum (devoid of NK1 receptors) ratios[11]. To evaluate whether this tracer can be used to quantitatively assess NK1 receptor status, tracer kinetic models were applied to the predosing data mentioned above. In addition, precision of these models was assessed using a test-retest study, providing an estimate of within-subject variability (%) for each method. The intraclass correlation coefficient (ICC) was used as a measure of reliability[28], as this statistical parameter compares within- to between-subject variability in repeated measurements.

After identifying that sticking of the tracer was a problem for generating reliable plasma curves, two potential correction algorithms were investigated. The first correction, previously validated for [11C]deprenyl, assumes that sticking can be described by a single exponential function[23]. It was clear, however, that this simple correction was not able to provide a satisfactory correction for sticking of [11C]R116301 to the walls of the online tubing, as no consistent match between online curve and manual samples could be obtained. Apparently, a single exponential did not fully characterise sticking kinetics and, therefore, a second attempt was made using a double exponential function. This approach, however, also was not successful, as most fits did not converge, indicating that too many parameters were involved. Consequently, it was not possible to use plasma input models.

As the cerebellum is known to be devoid of NK1 receptors[3], it was possible to investigate reference tissue models using cerebellum as reference tissue. In the present study, SRTM was preferred over FRTM in all but one of the baseline scans according to both Akaike and Schwarz criteria. In addition, standard errors of parameter estimates were much higher for FRTM than for SRTM. Therefore, SRTM was chosen as the (reference tissue) model of choice. Baseline scans showed a mean BP_{ND} of 0.63 ± 0.29 (n=19). As expected, specific binding reduced to almost zero after pre-dosing with 125 mg aprepitant in the blocking study (n=3), indicating almost complete blocking.

Test-retest results for SRTM derived BP_{ND} showed good reproducibility and excellent reliability. It should be noted, however, that there was little specific signal in subject 3 and none in subject 6. The absence of a specific signal in this latter subject was consistent in both test and retest scans, indicating that this is unlikely due to a measurement error. Both subjects were, however, screened and scanned in the same manner as all other subjects and all clinical parameters were within normal range.

The moderate age effect (7% decrease per decade, significant in other regions than striatum) of binding to NK1 receptors that has been reported[30] could potentially have played some role in the lower signal of subject number 3, as this was a male of 41. This would, however, be no explanation for subject number 6, as this was a male of 27 years old. Theoretically, it could also be due to differences in genetic make-up, for example a polymorphism in the binding region of the human NK1 receptor gene, potentially resulting in lower affinity for [11C]R116301. A recent study, however, reported that the variant (Y192H) displayed similar properties as the wild type receptor[31]. Therefore, at present there is no evidence for an affinity related change in binding of [11C]R116301 to the receptor based on receptor polymorphisms. Therefore, it still remains unclear as to why specific binding measured in subject number 6 was so low.

Finally, for potential application in routine clinical studies, SUVr–1, an index of binding potential, was investigated. Note, that this ratio would equal BP_{ND} in case of true equilibrium. Test-retest results for SUVr–1 were excellent, showing very low test-retest variability (6.2%) and excellent reliability (ICC: 0.98). In addition, the signal reduced to zero after predosing with aprepitant.

To assess the validity of a simplified SUVr–1 approach, results were compared with BP_{ND} obtained using SRTM. Using both blocking and test-retest data, a good correlation (R^2 = 0.96) was obtained. It should be noted, however, that SUVr–1 was consistently lower than BP_{ND} (slope = 0.8 in Figure 2), indicating absence of equilibrium. Nevertheless, the high correlation shown in figure 2 indicates that SUVr–1 might provide a reasonable estimate/index of BP_{ND} in case of clinical studies, although both figures 2 and 3 demonstrate that SUVr-1 underestimates BPnd for regions showing higher binding.

In conclusion, in the absence of a reliable plasma input function, SRTM is the method of choice for quantifying [11C]R116301 binding is SRTM, as reliability (ICC) was excellent and test-retest variability low. The semi-quantitative tissue ratio method (SUVr–1) holds promise in a clinical setting, showing high correlation with SRTM, but further studies are needed to assess its validity in patient populations.

References

1. K. Ebner and N. Singewald, "The role of substance P in stress and anxiety responses," *Amino Acids* 2006;31: 251-72.
2. R. Hargreaves, "Imaging substance P receptors (NK1) in the living human brain using positron emission tomography," *J.Clin.Psychiatry* 2002;63 Suppl 11: 18-24.
3. J. Hietala, M. J. Nyman, I. Eskola, A. Laakso, T. Gronroos, V. Oikonen, J. Bergman, M. Haaparanta, S. Forsback, P. Marjamaki, P. Lehikoinen, M. Goldberg, D. Burns, T. Hamill, W. S. Eng, A. Coimbra, R. Hargreaves, and O. Solin, "Visualization and quantification of neurokinin-1 (NK1) receptors in the human brain," *Molecular Imaging and Biology* 2005;7: 262-72.
4. M. S. Kramer, N. Cutler, J. Feighner, R. Shrivastava, J. Carman, J. J. Sramek, S. A. Reines, G. H. Liu, D. Snavely, E. Wyatt-Knowles, J. J. Hale, S. G. Mills, M. MacCoss, C. J. Swain, T. Harrison, R. G. Hill, F. Hefti, E. M. Scolnick, M. A. Cascieri, G. G. Chicchi, S. Sadowski, A. R. Williams, L. Hewson, D. Smith, E. J. Carlson, R. J. Hargreaves, and N. M. J. Rupniak, "Distinct mechanism for antidepressant activity by blockade of central substance P receptors," *Science* 1998;281: 1640-5.
5. M. S. Kramer, A. Winokur, J. Kelsey, S. H. Preskorn, A. J. Rothschild, D. Snavely, K. Ghosh, W. A. Ball, S. A. Reines, D. Munjack, J. T. Apter, L. Cunningham, M. Kling, M. Bari, A. Getson, and Y. Lee, "Demonstration of the efficacy and safety of a novel substance P (NK1) receptor antagonist in major depression," *Neuropsychopharmacology* 2004;29: 385-92.
6. S. McLean, "Nonpeptide antagonists of the NK1 tachykinin receptor," *Medicinal Research Reviews* 1996;16: 297-317.
7. C. De Felipe, J. F. Herrero, J. A. O'Brien, J. A. Palmer, C. A. Doyle, A. J. H. Smith, J. M. A. Laird, C. Belmonte, F. Cervero, and S. P. Hunt, "Altered nociception, analgesia and aggression in mice lacking the receptor for substance P," *Nature* 1998;392: 394-7.
8. S. W. Park, Y. P. Yan, I. Satriotomo, R. Vemuganti, and R. J. Dempsey, "Substance P is a promoter of adult neural progenitor cell proliferation under normal and ischemic conditions," *Journal of Neurosurgery* 2007;107: 593-9.
9. Y. Watanabe, H. Asai, T. Ishii, S. Kiuchi, M. Okarnoto, H. Taniguchi, M. Nagasaki, and A. Saito, "Pharmacological characterization of T-2328, 2-fluoro-4 '-methoxy-3 '-[[[(2S,3S)-2-phenyl-3-piperidinyl]amino]methyll][1,1 '-biphenyl]-4-carbonitrile dihydrochloride, as a brain-penetrating antagonist of tachykinin NK1 receptor," *Journal of Pharmacological Sciences* 2008;106: 121-7.
10. M. Van der Mey, C. G. M. Janssen, F. E. Janssens, M. Jurzak, X. Langlois, F. M. Sommen, B. Verreet, A. D. Windhorst, J. E. Leysen, and J. D. M. Herscheid, "Synthesis and biodistribution of [C-11]R116301, a promising PET ligand for central NK1 receptors," *Bioorganic & Medicinal Chemistry* 2005;13: 1579-86.
11. Wolfensberger, S. P. A., van Berckel, B. N. M., Araiksinen, A. J., Maruyama, K., Boellaard, R., Lubberink, M., Carey, W. D. H., Reddingius, W., Veltman, D. J., Windhorst, B. D., Leysen, J. E., and Lammertsma, A. A. "First evaluation of [^{11}C]R116301 as an in vivo tracer of NK1 receptors in man." *Molecular Imaging and Biology*. 2009. Jul-Aug;11(4):241-5. Epub 2009 Mar 31.
12. First MB, S. R. G. M. W. J. Structured clinical interview for DSM-IV clinical version (SCID-I/CV). American Psychiatric Press, Washington, D.C.
13. G. Brix, J. Zaers, L. E. Adam, M. E. Bellemann, H. Ostertag, H. Trojan, U. Haberkorn, J. Doll, F. Oberdorfer, and W. J. Lorenz, "Performance evaluation of a whole-body PET scanner using the NEMA protocol. National Electrical Manufacturers Association," *J.Nucl.Med.* 1997;38: 1614-23.
14. R. Boellaard, A. van Lingen, S. C. M. van Balen, B. G. Hoving, and A. A. Lammertsma, "Characteristics of a new fully programmable blood sampling device for monitoring blood radioactivity during PET," *European Journal of Nuclear Medicine* 2001;28: 81-9.
15. M. Defrise, P. E. Kinahan, D. W. Townsend, C. Michel, M. Sibomana, and D. F. Newport, "Exact and approximate rebinning algorithms for 3-D PET data," *IEEE Trans.Med.Imaging* 1997;16: 145-58.
16. F. Maes, A. Collignon, D. Vandermeulen, G. Marchal, and P. Suetens, "Multimodality image registration by maximization of mutual information," *IEEE Trans.Med.Imaging* 1997;16: 187-98.
17. J. West, J. M. Fitzpatrick, M. Y. Wang, B. M. Dawant, C. R. Maurer, Jr., R. M. Kessler, R. J. Maciunas, C. Barillot, D. Lemoine, A. Collignon, F. Maes, P. Suetens, D. Vandermeulen, P. A. van den Elsen, S. Napel, T. S. Sumanaweera, B. Harkness, P. F. Hemler, D. L. Hill, D. J. Hawkes, C. Studholme, J. B. Maintz, M. A. Viergever, G. Malandain, R. P. Woods, and ., "Comparison and evaluation of retrospective intermodality brain image registration techniques," *J.Comput.Assist.Tomogr.* 1997;21: 554-66.
18. M. Bergstrom, R. J. Hargreaves, H. D. Burns, M. R. Goldberg, D. Sciberras, S. A. Reines, K. J. Petty, M. Ogren, G. Antoni, B. Langstrom, O. Eskola, M. Scheinin, O. Solin, A. K. Majumdar, M. L. Constanzer, W. P. Battisti, T. E. Bradstreet, C. Gargano, and J. Hietala, "Human positron emission tomography studies of brain neurokinin 1 receptor occupancy by aprepitant," *Biological Psychiatry* 2004;55: 1007-12.

19.L. Caberlotto, Y. L. Hurd, P. Murdock, J. P. Wahlin, S. Melotto, M. Corsi, and R. Carletti, "Neurokinin 1 receptor and relative abundance of the short and long isoforms in the human brain," *European Journal of Neuroscience* 2003;17: 1736-46.

20.Zhang Y. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. Brady M. IEEE Trans Med Imaging, 2001 Jan;20(1):45-57. Smith S.

21.A. A. Lammertsma, C. J. Bench, S. P. Hume, S. Osman, K. Gunn, D. J. Brooks, and R. S. J. Frackowiak, "Comparison of methods for analysis of clinical [C-11]raclopride studies," *Journal of Cerebral Blood Flow and Metabolism* 1996;16: 42-52.

22.A. A. Lammertsma, C. J. Bench, G. W. Price, J. E. Cremer, S. K. Luthra, D. Turton, N. D. Wood, and R. S. J. Frackowiak, "Measurement of Cerebral Monoamine Oxidase-B Activity Using L-[C-11]Deprenyl and Dynamic Positron Emission Tomography," *Journal of Cerebral Blood Flow and Metabolism* 1991;11: 545-56.

23.S. P. Hume, R. Myers, P. M. Bloomfield, J. Opackajuffry, J. E. Cremer, R. G. Ahier, S. K. Luthra, D. J. Brooks, and A. A. Lammertsma, "Quantitation of Carbon-11-Labeled Raclopride in Rat Striatum Using Positron Emission Tomography," *Synapse* 1992;12: 47-54.

24.A. A. Lammertsma and S. P. Hume, "A simplified reference tissue model for PET receptor studies," *Journal of Nuclear Medicine* 1996;37: 1013.

25.R. B. Innis, V. J. Cunningham, J. Delforge, M. Fujita, A. Giedde, R. N. Gunn, J. Holden, S. Houle, S. C. Huang, M. Ichise, H. Lida, H. Ito, Y. Kimura, R. A. Koeppe, G. M. Knudsen, J. Knuuti, A. A. Lammertsma, M. Laruelle, J. Logan, R. P. Maguire, M. A. Mintun, E. D. Morris, R. Parsey, J. C. Price, M. Slifstein, V. Sossi, T. Suhara, J. R. Votaw, D. F. Wong, and R. E. Carson, "Consensus nomenclature for in vivo imaging of reversibly binding radioligands," *Journal of Cerebral Blood Flow and Metabolism* 2007;27: 1533-9.

26. H. Akaike, "New Look at Statistical-Model Identification," *Ieee Transactions on Automatic Control* 1974;AC19: 716-23.

27.G. Schwarz, "Estimating Dimension of A Model," *Annals of Statistics* 1978;6: 461-4.

28.M. Laruelle, "Modelling: when and why?," *European Journal of Nuclear Medicine* 1999;26: 571-2.

29.E. Salmi, S. Aalto, J. Hirvonen, J. W. Langsjo, A. T. Maksimow, V. Oikonen, L. Metsahonkala, J. Virkkala, K. Nagren, and H. Scheinin, "Measurement of GABA(A) receptor binding in vivo with [C-11] flumazenil: A test-retest study in healthy subjects," *Neuroimage* 2008;41: 260-9.

30.M. J. Nyman, O. Eskola, J. Kajander, T. Vahlberg, S. Sanabria, D. Burns, R. Hargreaves, O. Solin, and J. Hietala, "Gender and age affect NK1 receptors in the human brain - a positron emission tomography study with [F-18]SPA-RQ," *International Journal of Neuropsychopharmacology* 2007;10: 219-29.

31.G. P. Randolph, J. S. Simon, M. G. Arreaza, P. Qiu, J. E. Lachowicz, and R. A. Duffy, "Identification of single-nucleotide polymorphisms of the human neurokinin 1 receptor gene and pharmacological characterization of a Y192H variant," *Pharmacogenomics.J.* 2004;4: 394-402.

7

Summary and discussion

The research described in this thesis examined brain correlates of anxiety and depression using various neuroimaging techniques. Sections A and B report on functional and structural neuroimaging studies in MZ twins at high and low risk for anxiety and depression. In section C, a novel PET ligand for NK1 receptors, which are involved in anxiety and depression, was evaluated *in vivo*. This approach could be the basis of future imaging genetics studies focusing on functional, structural and molecular (receptor ligand) changes, to disentangle the contributions of genetic and environmental risk factors for anxiety and depression.

Summary sections A and B: the MZ discordant/concordant twin study

In sections A and B, the hypothesis was tested that genetic and environmental risk factors for anxiety and depression impact on partly different brain structures or, alternatively, even if they converge on the same brain structures, they do so in entirely different, perhaps opposite, ways.

To this end, dual modal imaging was used in MZ twin pairs strongly concordant or discordant for the risk for anxiety and depression to establish intrapair differences in regional brain structure and function. 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 [57], which were shown to have strong predictive value for clinical anxiety and depression, as assessed by the Composite International Diagnostic Interview[58].

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, the intrapair comparison of discordant twins highlighted neural activation and brain regions that are particularly susceptible to environmental factors. In MZ twin pairs that are selected to be highly concordant, the risk was either very high in both members of the pair (even though they have been exposed to partly different environments) or very low in both members. Comparing these two groups creates a genetic contrast, which was confirmed by verifying that the parents of these twins indeed had high versus low risk scores as well. Hence, the low / high group comparison of concordant twins highlighted neuronal activation and brain regions that are particularly susceptible to genetic factors.

Section A: Functional Neuroimaging

Previous functional brain imaging studies in subjects suffering from anxiety and depressive disorder have shown deviant amygdala responses to emotional stimuli, but compared to healthy controls both hyperactivity and hypoactivity have been reported. In **chapter 2**, the hypothesis was tested that these discrepant findings are based on different effects of genetic and environmental risk factors on amygdala functioning. To test this hypothesis, amygdala responses to an emotional faces paradigm were assessed during fMRI in monozygotic twin

pairs discordant for the risk of anxiety and depression (n=10 pairs) and in monozygotic twin pairs concordant for high (n=7 pairs) or low (n=15 pairs) risk for anxiety and depression. Main effects (all faces vs. baseline) revealed robust bilateral amygdala activity across groups. In discordant twins, increased amygdala responses were found for negatively valenced stimuli (angry/anxious faces) in high-risk twins compared to their low-risk co-twins. In contrast, concordant high-risk pairs revealed blunted amygdala reactivity to both positive and negative faces compared with concordant low-risk pairs. Post-hoc analyses showed that these findings were independent of serotonin transporter (5-HTTLPR) genotype.

These findings indicate that amygdala hyperactivity was found in subjects who are at high risk for anxiety and depression through environmental factors, but amygdala hypoactivity in those at risk mainly through genetic factors. The hyperactivity of the environmentally at risk twins disappeared after correcting for state anxiety, which indicates that hyperresponsiveness to emotional stimuli went together with increased anxiety. State anxiety was similarly increased in the twins at genetic risk, although here amygdala reactivity to the emotional stimuli was lower compared to neutral stimuli and this hypoactivity did not disappear when state anxiety was taken into account. This pattern of results appears to reflect higher baseline amygdala activation in subjects at high genetic risk for anxiety and depression, which acts to reduce further activation in response to general increases in state anxiety and specific emotion-inducing stimuli (e.g. angry faces).

In **chapter 3**, emotional processing and brain activation were examined during an encoding and recognition paradigm using emotionally salient words in a sample of monozygotic twin pairs at low and high risk for anxiety and depression, using the same study sample as in chapter 2. Psychological theories of major depression have emphasized the role of negative biases in information processing in the etiology and the maintenance of the disorder. Such biases have been reported both for interpretation and storage of emotional information[59-62]. These observations raise the possibility that emotional biases might pre-date the onset of clinical depression and thereby represent a risk factor for subsequent development of illness in predisposed individuals. To investigate emotional bias prior to onset of depression it is necessary to study people who are at risk for anxiety and depression, but who are not clinically depressed. To date, studies combining brain imaging and neuropsychological testing in groups at high risk for depression and anxiety, but not yet affected, have been scarce and have focused primarily on executive functioning.

In the present study, performance data did not support the existence of a negative response bias in high risk subjects. At the neural level, however, increased left inferior frontal gyrus activation by negative words was found in high risk subjects through either genetic or environmental risk factors, most prominently during recognition. In other words, genetic and environmental pathways seemed to converge, both pointing towards increased activation in the left inferior frontal gyrus in response to negatively valenced words in both high risk groups. This may indicate that increased activation of left inferior frontal gyrus in a verbal emotional memory task is a useful vulnerability marker for anxiety and depression.

Section B: Structural Neuroimaging

In **chapter 4**, the same study sample as in chapters 2 and 3 was used. Volume reductions in the temporal lobe were observed in high risk twins, most notably in the left posterior hippocampal region, but exclusively in twins at high risk through environmental factors. 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. This pattern of results suggests that damage to temporal lobe structures may be specific to an environmentally driven aetiology of anxiety and depression. The following potential explanations were considered for this pattern of findings. The high risk discordant twins reported exposure to more, and more severe, life stressors early in their lives. In line with the glucocorticoid cascade hypothesis, these stressors may well account for part of the environmental effects on temporal grey matter volume, especially the hippocampal region, having a high density of glucocorticoid receptors. An alternative explanation is epigenetic reprogramming. Epigenetic reprogramming can create large phenotypic divergence in genetically identical subjects by selectively repressing the expression of some genes. In particular in older twins, remarkable differences in their gene-expression profile have been found[63].

Epigenetic drift, therefore, is a potential source for discordance in amygdala activation, left temporal volumes and the risk for anxiety and depression. Finally, it might be possible that both explanations converge, and that life stress and hypothalamic-pituitary-adrenocortical (HPA)-axis activation somehow have a direct impact on epigenetic reprogramming. Future (longitudinal) studies should address this important question.

Discussion sections A and B

Several lines of evidence suggest the presence of specific neural circuits within the limbic cortical system that mediate stress responsiveness, mood and emotion regulation[64-66]. A recent comprehensive meta-analysis revealed brain volume reductions in depressed patients in many regions related to emotional processing and stress regulation, such as the frontal lobe and hippocampus, and amygdala[67]. In the studies described in this thesis both volumetric and functional abnormalities were found in those regions.

The hippocampus is well known to be a target for glucocorticoids, which potentially contribute to atrophy in this brain structure[68-79]. Furthermore, amygdala and prefrontal cortex are also involved in HPA-axis regulation. This is important to note, as depression has been associated with disrupted HPA-axis activity and increased cortisol levels[80,81], also mentioned in section B. Several mechanisms have been proposed to explain how prolonged stress can result in limbic and prefrontal (volumetric) abnormalities, such as decreased dendritic branching[82,83], loss of neurons, or decreased expression of brain derived neurotrophic factor[68,82,84]. Evidence for stress induced brain abnormalities in depression is also provided by studies examining genetic variations in the glucocorticoid receptor gene. Especially functional polymorphisms of the NR3C1 (nuclear receptor subfamily 3, group C, member 1) gene are associated with increased susceptibility to depression[68,85,86]. Interestingly, a recent study reported an association of four illness-related polymorphisms of the NR3C1 gene with overall smaller hippocampal volumes in patients with depression[87]. This suggests that risk alleles of the NR3C1 gene influence hippocampal volume, and it would not be surprising when future research will reveal more risk alleles associated with volume changes or differences in activation, potentially confounding study results with un unknown effect size.

A genetic variant that repeatedly has been implicated in amygdala function is the 5-HTTLPR promoter polymorphism. The short allele (S) of the promoter polymorphism is associated with reduced expression of the gene and lower availability of the transporter[88,89], and individuals carrying the s-allele tend to have increased anxiety related temperamental traits, which in turn are related to increased risk of developing depression[68,90]. The few studies that examined the association between 5-HTTLPR polymorphism and brain volumes in depressed patients showed mixed results with respect to amygdala and hippocampus[93-96], but fMRI studies systematically have shown that this polymorphism affects amygdala activity in response to emotional stimuli[38,89,91]. This latter finding was not replicated in the study described in this thesis, but sample size may have been a major issue.

Frontal regions (anterior cingulate, orbitofrontal and prefrontal cortex) are known to exert emotion regulation by inhibiting the activity of limbic regions such as the hippocampus and the amygdala[97,98]. Prominent volume reductions in those frontal regions in major depression were demonstrated[68] in many studies. Although these findings were not replicated, evidence for different functional activation in the left inferior frontal gyrus during emotional word processing was shown.

Limitations of the MRI twin study design

When studying the effects of anxiety and depression on MRI traits, four important effects need to be disentangled: the effects of an ongoing depressive episode, the effects of anti-depressant medication, the effects of vulnerability traits and the effects of increased anxiety at the time of scanning. The main intent was to detect effects of trait vulnerability caused either by detrimental environmental or genetic risk factors, which meant that the other three (confounding) effects needed to be contained as much as possible. It was possible to limit the effects of current depression and antidepressant medication. Apart from one exception in a concordant high-risk pair, no twin was diagnosed with current MDD at the time of scanning, and only one subject used antidepressants (results did not noticeably differ after excluding these subjects). However, there clearly were higher levels of state anxiety and depressive symptoms scores at the time of scanning in the (trait) high risk twins. Increased left inferior frontal gyrus activity during emotional words may therefore reflect state anxiety at the time of scanning rather than genetic or environmental effects on this brain region. Although this first limitation is recognised, it is very hard to envision a design that successfully separates state and trait anxiety.

A second limitation is the absence of baseline activation measurements. A prerequisite of finding a significant BOLD signal is that task related activation in a region of interest is significantly different from the reference baseline activation in that region, as fMRI is based on relative measurements. However, it is likely that the response to stimuli is simply based on ceiling effects, as the response is dependent on the baseline activation[99]. Therefore, interpreting genetic and environmental effects on regional fMRI contrasts should ideally be done in relation to parallel genetic and environmental effects on baseline activation of those regions.

Conclusions sections A and B

In conclusion, the main finding of these studies was that genetic and environmental risk factors for anxiety and depression may impact on partly different brain structures (amygdala, hippocampus), but that they could also converge on the same brain structures (frontal lobe). Therefore, future brain imaging studies on anxiety and depression should aim to avoid an admixture of subjects who are at genetic risk with those at risk for environmental reasons. This can, in principle, be done by selecting patients with known exposure to strong environmental risk factors (e.g. childhood trauma) or with known genetic risk markers. Based on the current understanding of the role of genetics in psychiatric disorders, however, the discordant/concordant MZ twin design remains the most powerful method of separating genetic and environmental risk factors. If fMRI is used as the imaging modality, however, an active attempt should be made to measure baseline activation levels, for example by

using $^{15}OH_2O$ PET for absolute measurements of cerebral perfusion or arterial spin labelling techniques.

Summary section C: Molecular Neuroimaging

NK1 receptors have been implicated in various neuropsychiatric and other disorders, and NK1 receptors are potentially important targets for drug development, as they have been implicated in depression, anxiety and pain perception. R116301 is a selective high affinity NK1 receptor antagonist. In **chapter 5**, a pilot study was performed, evaluating [^{11}C]R116301 as a potential PET ligand for the NK1 receptor. More specifically, the presence of a specific signal was investigated using two dynamic 90 minutes [^{11}C]R116301 scans, separated by 5 hours, in 3 normal volunteers, before and after an oral dose of 125 mg aprepitant. Data were analysed using striatum to cerebellum standardised uptake value (SUV) ratios. Baseline SUV ratios at 60-90 minutes after injection ranged from 1.22 to 1.70. Following aprepitant administration this specific signal was completely blocked. Aprepitant administration did not significantly affect cerebellar uptake, confirming the absence of NK1 receptors in cerebellum. These preliminary results indicated that [^{11}C]R116301 has potential as a radioligand for *in vivo* assessment of NK1 receptors in the human brain.

As a specific signal was found in chapter 5, **chapter 6** describes the evaluation of various quantitative outcome measures of [^{11}C]R116301 binding in healthy subjects using both blocking and test-retest data with the aim to find the optimal model for quantification. Two dynamic 90 minutes [^{11}C]R116301 scans, separated by 5 hours, were performed in 11 healthy volunteers. Data from the blocking study, using the same 3 paired PET studies as in chapter 5, were now used for further evaluation, whilst in the other 8 no intervention was performed (i.e. test-retest). Whole striatum, as defined on a co-registered MRI scan, was used as tissue of interest, as it has the highest density of NK1 receptors. Cerebellum was used as reference tissue. Unfortunately, measured plasma curves were not reliable due to severe stickiness of the tracer. Even after implementing a validated sticking correction, no useful results were obtained when plasma input models were used. In contrast, reference tissue models appeared to be more stable with the simplified reference tissue model (SRTM) performing best. Average (± SD) SRTM derived mean binding potential BP_{ND} of all (first) baseline scans was 0.64 ± 0.31, which reduced to −0.01 ± 0.03 following aprepitant administration. Test-retest results showed good variability (14.0 ± 10.7%) and excellent reliability (intraclass correlation coefficient ICC = 0.93) for SRTM derived BP_{ND}. Striatum to cerebellum SUV ratios minus 1, an approximation of BP_{ND}, showed excellent variability (6.2 ± 3.1%) with excellent reliability (0.98) and correlated well with SRTM (r^2 = 0.96) with acceptable (15%) bias. In conclusion, SRTM was found to be the optimal model for quantification of [^{11}C]R116301 binding, but semi-quantitative SUV methods hold promise for routine clinical applications.

Discussion section C

Subsance P (SP) was first discovered by Ulf von Euler and John Gaddum in 1931[100]. Gaddum and Schild[101] named this new agent SP, P referring to the powder which was obtained after the extraction procedure from equine brain and gut, and which was found to have potent hypotensive and smooth muscle contractile properties.

To date, almost 8 decades later, the neuropeptide SP and its preferred receptor, the tachykinin neurokinin type 1 (NK1) receptor, have been of particular interest because of their potential implication in various neuropsychiatric and other disorders. Depression and anxiety, for example, are related to changes in the release of SP binding to NK1 receptors in the human brain[102-106]. However, despite extensive preclinical and clinical studies on the widely distributed central pathways and NK1 receptors in pain, anxiety, depression, schizophrenia, Parkinson's disease, and Alzheimer's disease and other neuro-psychiatric disorders, to date, NK1 receptor antagonists have only found proven efficacy in the prevention of acute and delayed chemotherapy induced nausea and vomiting.

The NK1 antagonist, aprepitant (EMEND®) is on the market for treatment of radio- and chemotherapy induced emesis. Recently, compounds like casopitant and AV608 have entered phase II clinical trials for various indications such as myalgia, insomnia, irritable bowel syndrome and urinary incontinence. As there is very little known about the functions mediated by SP in humans, PET imaging of NK1 receptors is a useful tool to study this system in health and psychiatric and neurological diseases. Moreover, it could be a useful tool to visualise and quantify specific receptor binding in order to gain more insight in neurotransmitter-receptor function and it could aid in assessing the optimal dose range of new drugs that act by occupancy of central NK1 receptors. For this purpose, quantification of [^{11}C]R116301 binding to the NK1 receptor was evaluated in section C.

Conclusions section C

It can be concluded from these PET studies, that SRTM[107] is the optimal model for quantification of [^{11}C]R116301 binding. This method shows good test-retest variability and good reliability, and it seems feasible to shorten scan durations. Tissue ratio methods hold promise for clinical applications as even shorter scan durations can be used. In future studies, tissue ratio methods need to be further validated over a larger range of BP_{ND}. Furthermore, it will be necessary to demonstrate a difference in BP_{ND} between healthy controls and patients. In addition, it should be evaluated whether the good correlation with SRTM observed in normal controls also applies to various patient populations, for example those who are at risk for anxiety and depression, and those who are diagnosed with major depression. In other words, this should be performed by comparing groups with abnormal NK1 receptor status with healthy controls.

At present, there is no ideal PET tracer for NK1 receptors. Of the available tracers [^{18}F]SPARQ shows a much higher specific signal than [^{11}C]R116301. Therefore, at least in theory, it should provide a more accurate assessment of NK1 receptor status or occupancy, which could be important dealing with small differences between groups. For example, the expected reduction in binding of NK1 receptors in anxiety and depression (12-21% panic disorder) can better be studies using [^{18}F]SPARQ, as far fewer patients would be required. Nevertheless, for clinical applications and also for research purposes,[^{18}F]SPARQ is hampered by its slow kinetics, requiring very long study durations, thereby increasing the risk of movement artefacts. At the cost of a reduced signal,[^{11}C]R116301 has the advantage that studies can be performed within a reasonable time (90 minutes).[^{11}C]R116301 could have a future role in assessing whether a (new) drug results in significant NK1 receptor occupancy.
When ranking the affinity of NK1 ligands, it is clear that the affinity of R116301 is lower than that of SPARQ, which is in agreement with the higher specific signal observed with [^{18}F]SPARQ On the other hand, the lower affinity of R116301 implies that [^{11}C]R116301 could be more sensitive to differences in endogenous substance P concentrations. This would be very interesting in intervention studies, where release of the endogenous ligand could be manipulated by a pharmacological challenge or, for example, a fear stimulus. In fact, [^{11}C]R116301 should be ideally suited for this, as it has a lower affinity than any other (known) available alternative tracer for the NK1 receptor. Clearly, further studies are needed to establish whether this is feasible.

Future perspectives

This thesis has shown the power of a discordant/concordant MZ twin design to detect, using MRI, structural and functional brain differences in subjects at genetically and/or environmentally determined low and high risk for anxiety and depression. Future studies may apply this fMRI and molecular imaging design to other neuropsychiatric illnesses based on a combined genetic and environmental aetiology. First, the issue of differences in baseline activation between groups and within an MZ twin pair could be addressed by making use of either [^{15}O]H_2O PET for absolute measurements or arterial spin labelling (MRI) as an imaging tool for cerebral perfusion. This will allow comparison of baseline activation states within twin pairs to possibly explain differences in neuronal activation measured, using fMRI. One of the drawbacks of arterial spin labelling is its inability to explore the whole brain. It may, nevertheless, be suitable to study one specific region of interest.
Secondly, further use of PET could clarify differences in genetic and environmental risk factors on a molecular (e.g. receptor) level and, therefore, help with interpreting results of functional and structural neuroimaging studies in the same subjects. Various ligands for neuroreceptor mapping can be used. For example, serotonin transporter (SERT) status can be investigated using [^{11}C] DASB, a ligand to the serotonin transporter receptor. This can be used to explore possible differences in receptor status between genetically and environmentally determined risk factors. Therefore, receptor density and affinity of the ligand to the receptor in specific brain regions could shed some light on the functional and structural imaging data, i.e. increased activation or volume reduction in a region of interest could be associated with a change in receptor status in this region.
As mentioned in the discussion of sections A and B, genetic and environmental risk factors could lead to differential underlying neurobiological mechanisms, such as differences in receptor status. These differences (different types of vulnerability profiles for the specific disorder) at a receptor level may have differential responses to pharmacologic intervention (treatment). Unravelling these underlying mechanisms in future research could, therefore, lead to novel or more individualised therapeutic or even preventive strategies.

References

57. Boomsma DI, Beem AL, van den BM *et al*. Netherlands twin family study of anxious depression (NETSAD). *Twin Res* 2000; 3:323-34.
58. Middeldorp CM *et al*. The association of personality with anxious and depressive psychopathology. T Canli, editor. The Biological Basis of Personality and Individual Differences. *New York: Guilford Press* 2006; 251-272.
59. Bradley BP, Mogg K, Williams R. Implicit and Explicit Memory for Emotion-Congruent Information in Clinical Depression and Anxiety. *Behaviour Research and Therapy* 1995; 33:755-70.
60. Leppanen JM. Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. *Current Opinion in Psychiatry* 2006; 19:34-9.
61. Murphy BC, Shepard SA, Eisenberg N, Fabes RA, Guthrie IK. Contemporaneous and longitudinal relations of dispositional sympathy to emotionality, regulation, and social functioning. *Journal of Early Adolescence* 1999; 19:66-97.
62. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biological Psychiatry* 2003; 54:515-28.
63. Fraga MF, Ballestar E, Paz MF *et al*. Epigenetic differences arise during the lifetime of monozygotic twins. *Proceedings of the National Academy of Sciences of the United States of America* 2005; 102:10604-9.
64. Tekin S, Cummings JL. Frontal-subcortical neuronal circuits and clinical neuropsychiatry: An update. *Journal of Psychosomatic research* 2002; 53:647-654.
65. Seminovicz DA, Mayberg HS, McIntosh AR *et al*. Limbic-frontal circuitry in major depression: A path modeling metanalysis. *Neuroimage* 2004; 22:409-418
66. Brody AL, Barsom MW, Botal RG *et al*. Prefrontal-subcortical and limbic circuit mediation of major depressive disorder. *Seminars in Clinical Neuropsychiatry* 2001; 6:102-12
67. Koolschijn PC, van Haren NE, Lensvelt-Mulders GJ *et al*. Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. *Human Brain Mapping* 2009; 30:3719-35.
68. Czeh B, Lucassen PJ. What causes the hippocampal volume decrease in depression? Are neruogenesis, glial changes and apoptosis implicated? *European Archives of Psychiatry and Clinical Neuroscience* 2007; 257:250-60.
69. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Archives of General Psychiatry*. 2000; 57:925-35.
70. Henn FA, Vollmayr B. Neurogenesis and depression: Etiology or epiphenomenon? *Biological Psychiatry* 2004; 56: 146-150.

71. Mcewen BS. Protection and damage from acute and chronic stress - Allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Biobehavioral Stress Response: Protective and Damaging Effects* 2004; 1032:1-7.
72. Mcewen BS. Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. *Metabolism-Clinical and Experimental* 2005; 54:20-3.
73. Mcewen BS, Olie JP. Neurobiology of mood, anxiety, and emotions as revealed by tudies of a unique antidepressant: tianeptine. *Molecular Psychiatry* 2005; 10:525-37.
74. Mcewen BS. Stressed or stressed out: What is the difference? *Journal of Psychiatry & Neuroscience* 2005; 30:315-8.
75. Mcewen BS. Stress, sex and the hippocampus: Protection, damage and aging. *International Journal of Neuropsychopharmacology* 2006; 9:S61.
76. Mcewen BS, Liston C, Morrison JH. Stress-induced structural plasticity in prefrontal cortex, amygdala and hippocampus. *Neuropsychopharmacology* 2006; 31:S13.
77. Mcewen BS. Physiology and neurobiology of stress and adaptation: Central role of the brain. *Physiological Reviews* 2007; 87:873-904.
78. Mcewen BS, Milner TA. Hippocampal formation: Shedding light on the influence of sex and stress on the brain. *Brain Research Reviews* 2007; 55:343-55.
79. Mcewen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *European Journal of Pharmacology* 2008; 583:174-85.
80. Bao AM, Meynen G, Swaab DF. The stress system in depression and neurodegeneration: focus on the human hypothalamus. *Brain Research Reviews* 2008; 57:531-53.
81. Swaab DF, Bao AM, Lucassen PJ. The stress system in the human brain in depression and neurogeneration. *Ageing Research Reviews* 2005; 4:141-94.
82. Radley JJ, Morrison JH. Repeated stress and structural plasticity in the brain. *Ageing Research Reviews* 2005; 4:271-87
83. Duman RS. Depression: a case of neuronal life and death? *Biological Psychiatry* 2004; 1;56:140-5.
84. Duman RS. Molecular and cellular theory of depression. *Archives of General Psychiatry*. 1997; 54:597-606
85. Van Rossum EF, Binder EB, Majer M *et al*. Polymorphisms of the glucocorticoid receptor gene and major depression. *Biological Psychiatry* 2006; 15;59:681-8
86. van West D, Van Den EF, Majer M *et al*. Glucocorticoid receptor gene-based SNP analysis in patients with recurrent major depression. *Neuropsychopharmacology* 2006; 31:620-7
87. Zobel A, JEssen F, Von WO *et al*. Unipolar depression and hippocampal volume: Impact of DNA sequence variants of the glucocorticoid receptor gene. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 2008; 147B:836-43

88. Lesch KP, Bengel D, Heils A *et al*. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; 274:1527-31
89. Durston S, de Zeeuw P, Staal WG. Imaging genetics in ADHD: A focus on cognitivecontrol. *Neuroscience and Biobehavioral Reviews* 2009; 33:674-89
90. Lotrich FE, Pollock BG. Meta-analysis of serotonin transporter polymorphisms and affective disorders. *Psychiatric Genetics* 2004; 14:121-9
91. Munafo MR, Brown SM, Hariri AR. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biological Psychiatry* 2008;63:852-7
92. Fitzgerald PB, Laird AR, Maller J *et al*. A meta-analytic study of changes in brain activation in depression. *Human Brain Mapping* 2008; 29:683-95
93. Ohara R, Schroder CM, Mahadevan R *et al*. Serotonin transporter polymorphism, memory and hippocampal volume in the elderly:Association and interaction with cortisol. *Molecular Psychiatry* 2007; 12:544-55
94. Frodl T, Meisenzahl EM, Zill P *et al*. Reduced hippocampal and amygdala changes in patients with major depressive disorder and healthy controls during a 1-year follow-up. *Journal of Clinical Psychiatry* 2004a; 65:492-9
95. Hickie IB, Naismith S, Ward PB *et al*. Reduced hippocampal volumes and memory loss in patients with early- and late-onset depression. British Journal of Psychiatry 2005; 186:197-202.
96. Taylor WD, Steffens DC, Payne ME *et al*. Influence of serotonin transporter promoter region polymorphisms on hippocampal volumes in late-life depression. *Archives of General Psychiatry* 2000; 62:537-44
97. Beauregard M, Levesque J, Bourgouin P. Neural correlates of conscious self-regulation of emotion. *Journal of Neuroscienc.* 2001; 21:RC165
98. Hariri AR, Bookheimer SY, Mazziotta JC. Modulating emotional responses: Effects of neocortical network on the limbic system. *Neuroreport* 2000; 11:43-48
99. Lacey JI. The evaluation of autonomic responses: toward a general solution. *Annals of the New York Academy of Sciences* 1956; 67:125-63
100. V Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. *The Journal of Physiology* 1931; 72 (1): 74–87
101. Gaddum JH, Schild H. Depressor substances in extracts of intestine. *The Journal of Physiology* 1934; 83 (1):1-14
105. Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides* 2000; 34:272-280.
103. Carpenter L.L, Bayat L, Moreno F *et al*. Decreased cerebrospinal fluid concentrations of substance P in treatment-resistant depression and lack of alteration after acute adjunct vagus nerve stimulation therapy. *Psychiatry Research* 2008; 157: 123-129

104. Griebel G. Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? *Pharmacology & Therapeutics* 1999; 82:1-61.
105. Kramer M.S, Cutler N, Feighner J. *et al*. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 1998; 281:1640-1645.
106. Kramer M.S, Winokur A, Kelsey, J. *et al*. Demonstration of the efficacy and safety of a novel substance P (NK1) receptor antagonist in major depression. *Neuropsychopharmacology* 2004; 29:385-392.
107. Lammertsma AA, Hume SP. A simplified reference tissue model for PET receptor studies. *Journal of Nuclear Medicine* 1996; 37:1013.

8

Nederlandse samenvatting

Dit proefschrift beschrijft de resultaten van onderzoek naar hersengebieden die betrokken zijn bij angst en depressie met behulp van verschillende beeldvormende (neuroimaging) technieken. De gebruikte technieken worden hier kort toegelicht. Om functionele en structurele verschillen in de hersenen zichtbaar te maken werd gebruikt gemaakt van zowel functionele- als structurele magnetic resonance imaging (MRI), dat wil zeggen beeldvorming van de hersenen, waarbij gebruik wordt gemaakt van magnetische resonantie. Functionele MRI (fMRI) is een speciale MRI techniek, die gebruik maakt van zeer kleine regionale veranderingen in de bloeddoorstroming, welke wordt veroorzaakt door neuronale activiteit in het betreffende hersengebied tijdens het maken van een neuropsychologische taak.

Om de binding van bepaalde stoffen (liganden) aan specifieke receptoren in de hersenen te kwantificeren werd gebruik gemaakt van positron emissie tomografie (PET). Bij deze techniek wordt een zeer kleine hoeveelheid van de stof radioactief gelabeld met een positron emitter en, na intaveneuze injectie, worden opname en klaring in de hersenen afgebeeld en gemeten met een PET scanner. Door toepassing van geschikte tracer modellen kan vervolgens de binding van deze ligand (tracer) aan de receptor gekwantificeerd worden.

In sectie A en B van dit proefschrift worden de functionele en structurele neuroimaging studies bij eeneiïge of wel monozygote (MZ) tweelingen met hoog en laag risico voor angst en depressie besproken. In sectie C, wordt een nieuwe PET ligand voor neurokinine1(NK1)-receptoren *in vivo* onderzocht. De NK1 receptor is betrokken bij angst en depressie.

Deze benadering kan de basis vormen voor toekomstig beeldvormend genetisch (imaging genetics) onderzoek gericht op functionele, structurele en moleculaire (receptor ligand) veranderingen, om op die manier de bijdrage van genetische- en omgevingsrisicofactoren voor angst en depressie te ontwarren.

Samenvatting secties A en B

De MZ discordant/concordant tweelingstudie

In sectie A en B, werd de hypothese getest of genetische- en omgevingsrisicofactoren voor angst en depressie a) van invloed zijn op gedeeltelijk verschillende hersenstructuren en/of b) in het geval dat deze risicofactoren van invloed zijn op dezelfde hersenstructuren, dit wordt bereikt op een geheel andere, wellicht tegengestelde, manier.

Daartoe werden twee imaging technieken gebruikt in MZ tweelingparen sterk concordant of discordant voor het risico op angst en depressie om verschillen in structuur en functie aan te tonen tussen de tweelingparen. Dit risico op angst en depressie werd berekend op basis van longitudinale onderzoeksgegevens over angst, depressie, neuroticisme en somatische angst, verzameld in 1991, 1993, 1997 en 2000. Eerder werd een sterk voorspellende waarde op klinische angst en depressie aangetoond op basis van deze onderzoeksgegevens, die voortkwamen uit het Composite International Diagnostic Interview.

Omdat MZ tweelingparen als genetisch identiek beschouwd kunnen worden, zou hun risico voor angst en depressie moeten worden veroorzaakt door differentiële blootstelling aan omgevingsinvloeden. Hieruit vloeit voort dat de intrapair vergelijking van de discordante tweelingen verschillen laat zien in neuronale activiteit en hersenstructuur, die gevoelig zijn voor omgevingsfactoren. Er werden in totaal 10 MZ tweelingparen geselecteerd, discordant voor het risico op angst en depressie.
Bij de MZ tweelingparen die geselecteerd werden als hoog of laag concordant, was het risico op angst en depressie ofwel zeer hoog in beide leden van het paar (zelfs al waren ze blootgesteld aan verschillende omgevingsfactoren) ofwel zeer laag in beide leden. Het vergelijken van deze twee groepen creëert een genetische contrast, hetgeen werd bevestigd door te controleren of de ouders van deze tweelingen inderdaad ook hoog versus laag risico scores hadden. Vandaar dat de laag-/hoog-groepsvergelijking van concordante tweelingen de verschillen laat zien in neuronale activiteit en hersenstructuur, die gevoelig zijn voor genetische factoren. Er werden in totaal 10 concordant hoge en 15 concordant lage MZ tweelingparen geselecteerd.

Sectie A: Functionele Neuroimaging

Eerdere functionele neuroimaging studies lieten een veranderde activatie van de amygdala op emotionele stimuli zien, waarbij zowel hyperactiviteit als hypo-activiteit werd gerapporteerd bij angst en depressie patiënten in vergelijking met gezonde controles. In **hoofdstuk 2**, werd de hypothese getest of deze tegenstrijdige bevindingen zijn gebaseerd op het verschil in effect van genetische- en omgevingsrisicofactoren op het functioneren van de amygdala. Om deze hypothese te testen, werd de amygdala reactie op een emotionele gezichtentaak bepaald tijdens fMRI in MZ tweelingparen die discordant, concordant hoog of concordant laag zijn, voor het risico op angst en depressie. Tijdens de taak werden er verschillende emotioneel beladen gezichten getoond, nl angstige, boze, blije en neutrale gezichten, afgewisseld met een baseline. Het hoofdeffect (alle gezichten versus baseline) van de taak liet een robuuste bilaterale amygdala-activiteit zien over alle groepen. Er werd een toegenomen amygdala response gevonden op negatieve stimuli (boos/angstige gezichten) in de discordant tweelingen met hoog risico op angst en depressie in vergelijking met hun discordant co-tweelingen met een laag risico. In tegenstelling hiermee lieten concordant hoge paren een afgestompt of 'blunted' amygdala reactiviteit zien op zowel positieve als negatieve gezichten in vergelijking met concordant lage paren. Post-hoc analyses toonden aan dat deze bevindingen onafhankelijk waren van het serotonine transporter (5-HTTLPR) genotype.
Deze bevindingen wijzen erop dat de amygdala hyperactiviteit werd gevonden bij deelnemers met een hoog risico op angst en depressie gebaseerd op omgevingsfactoren, terwijl amygdala hypoactiviteit juist werd gevonden bij deelnemers die een hoog risico op angst en depressie hadden voornamelijk op basis van genetische factoren. De hyperactiviteit van de tweelingen met verhoogd risico op basis van omgeving verdween na correctie voor het angstniveau ('state anxiety'), wat aangeeft dat hyperreactiviteit door emotionele stimuli samengaat met een verhoogde staat van angst. Het angstniveau was eveneens verhoogd in de tweelingen met een hoog risico op angst en depressie op basis van een genetisch risico. Echter, de reponse van de amygdala op emotionele stimuli ten op zichte van neutrale stimuli was bij deze groep lager en deze hypoactiviteit verdween ook niet nadat met state anxiety rekening was gehouden. Deze resultaten zouden kunnen duiden op een verhoogde baseline activiteit van de amygdala bij deelnemers met een hoog genetisch risico op angst en depressie, welke zou kunnen dienen om verdere activatie ten gevolge van een algemene verhoging van het angstniveau of op basis van een specifieke emotionele stimulus (boos gezicht) te verminderen.

In **hoofdstuk 3**, werd emotionele verwerking en hersenactiviteit onderzocht tijdens een coderings- en herkenningstaak met behulp van emotioneel geladen woorden (negatief, neutraal, positief en een baseline) in dezelfde onderzoeksgroep als in hoofdstuk 2. Psychologische theorieën over ernstige depressie hebben de rol benadrukt van een negatieve bias in de informatie-verwerking in de etiologie en het beloop van de aandoening. Een dergelijke emotioneel negatieve bias wordt zowel gezien bij de interpretatie als bij de opslag van emotionele informatie. Deze bevinding zou mogelijk kunnen wijzen op het bestaan van een emotionele bias voorafgaand aan de ontwikkeling van een klinische depressie en op die manier gezien kunnen worden als een risicofactor voor de verdere ontwikkeling van deze ziekte. Om te onderzoeken of de emotionele bias vooraf gaat aan de ontwikkeling van een depressie is het noodzakelijk om mensen te onderzoeken met een verhoogd risico op angst en depressie, maar die geen depressie of angst diagnose hebben. Tot op heden zijn functionele MRI studies, waarbij de deelnemer een bepaalde neuropsychologische test uitvoert tijdens het scannen, schaars, in groepen met een hoog risico op depressie en angst. De studies die beschikbaar zijn hebben zich voornamelijk gericht op het uitvoerende ofwel executieve functioneren.
In de studie beschreven in dit hoofdstuk, boden de performance data geen ondersteuning voor het bestaan van een negatieve emotionele respons bias in deelnemers met een hoog risico op angst en depressie. Op neuronaal niveau, echter, werd verhoogde activiteit gevonden in de linker frontale gyrus inferior op negatieve woorden bij deelnemers met een hoog risico, hetzij door middel van genetische-, hetzij door middel van omgevingsfactoren. Dit was het meest opvallend tijdens de herkenningstaak. Met andere woorden, genetische en omgevingsfactoren leken te convergeren. Dit kan betekenen dat verhoogde activering van de linker frontale gyrus inferior in een herkenningstaak met behulp van emotioneel geladen woorden, gebruikt zou kunnen worden als een kwetsbaarheidsmarker voor angst en depressie, ongeacht de onstaanswijze.

Deel B: Structurele Neuroimaging

In **hoofdstuk 4** werd bij tweelingen met een hoog risico op angst en depressie een volume vermindering waargenomen in de temporaalkwab, met name in de linker posterior hippocampale regio. Echter, dit werd uitsluitend gevonden bij tweelingen met een hoog risico door omgevingsfactoren. Een

groepsvergelijking tussen hoog en laag concordante paren, welke meer verschillen in genetische kwetsbaarheid weergeeft, liet geen volume vermindering zien in de temporaalkwab en de hippocampus posterior in de paren met een hoog risico op angst en depressie. Deze resultaten geven aan dat schade aan de structuren van de temporaalkwab specifiek zouden kunnen zijn voor een omgevingsgedreven pathogenese van angst en depressie. De volgende mogelijk verklaringen voor deze resultaten werden overwogen. De discordante tweelingen met hoog risico voor angst en depressie rapporteerden vaker, en ernstiger blootstelling aan life stressors vroeg in het leven. In lijn met de glucocorticoid(stresshormoon)-cascade-hypothese, zouden deze life stressors zeer wel verantwoordelijk kunnen zijn voor een deel van de omgevingseffecten op de grijze stof van de temporaal kwab, met name de hippocampus regio, welke een hoge dichtheid aan glucocorticoid receptoren heeft. Een alternatieve verklaring is epigenetische herprogrammering. Epigenetische herprogrammering kan in genetisch identieke tweelingen leiden tot grote fenotypische divergentie door het selectief onderdrukken of stimuleren van expressie van bepaalde genen. Er zijn opmerkelijke verschillen in gen-expressie profiel gevonden in met name oudere tweelingen.

Epigenetische drift is dus een potentiële bron voor het ontstaan van discordantie in de amygdala activiteit, de volumina van de linker temporaal kwab en het risico op angst en depressie. Ten slotte is het mogelijk dat beide verklaringen convergeren en dat life stress en de hypothalamus-hypofyse-bijnierschors (HPA)-as activatie op een of andere manier een directe impact hebben op epigenetische herprogrammering. Toekomstige (longitudinale) studies zouden gericht kunnen zijn op deze belangrijke vragen.

Samenvatting sectie C

Moleculaire Neuroimaging

NK1-receptoren zijn betrokken bij diverse neuropsychiatrische en andere aandoeningen, en zijn potentieel belangrijke targets voor de ontwikkeling van geneesmiddelen, omdat ze een rol spelen in o.a. angst, depressie en pijn perceptie.

R116301 is een selectieve antagonist met hoge affiniteit voor de NK1 receptor. In **hoofdstuk 5** is een verkennende studie (pilot) uitgevoerd bij drie gezonde vrijwilligers, ter evaluatie van [^{11}C]R116301 als een potentiële PET ligand voor de NK1 receptor, waarbij met name de aanwezigheid van een specifiek signaal werd onderzocht. Dit werd gedaan met behulp van twee dynamische [^{11}C]R116301 scans met een duur van 90 minuten. De tweede scan bij dezelfde vrijwilliger vond 5 uur na de start van de eerste scan plaats, vóór en na een orale dosis van 125 mg aprepitant, waarmee het specifieke signaal geblokkeerd zou moeten worden. De data uit deze scansessies werden geanalyseerd door de ratio te nemen van de opname in het striatum en het cerebellum (de kleine hersenen), met behulp van gestandaardiseerde waardes voor opname (SUV). Het striatum is de regio met de hoogste dichtheid aan NK1 receptoren. Het cerebellum is juist een regio zonder NK1 receptoren, en kan om die reden als referentie regio fungeren. Baseline (scan zonder interventie) SUV ratios op 60-90 minuten na injectie varieerden van 1,22 tot 1,70. Na toediening van aprepitant werd volledige blokkade van dit specifieke signaal bereikt. De toediening van aprepitant had geen significante invloed op de opname in het cerebellum, hetgeen de afwezigheid van NK1-receptoren in het cerebellum bevestigd. Deze voorlopige resultaten geven aan dat [^{11}C]R116301 mogelijkheden biedt als een radioligand voor de *in vivo* evaluatie van NK1-receptoren in de humane hersenen.

Omdat een specifiek signaal werd gevonden in hoofdstuk 5, beschrijft **hoofdstuk 6** de evaluatie van verschillende kwantitatieve uitkomstmaten van [^{11}C]R116301 binding in gezonde vrijwilligers met behulp van zowel de data van de blokkade studie als de data van een test-retest studie met als doel het meest geschikte model voor kwantificatie te vinden. Zoals eerder beschreven werden er twee dynamische [^{11}C]R116301 scans uitgevoerd met een duur van 90 minuten, waarbij de tweede scan 5 uur na de start van de eerste scan plaatsvond in 11 gezonde vrijwilligers. De data uit hoofdstuk 5, de eerste 3 gepaarde PET scans uit de blokkade studie (vóór en na een orale dosis van 125 mg aprepitant) werden gebruikt voor verdere analyse, terwijl bij de andere 8 gepaarde PET scans geen interventie werd uitgevoerd (test-retest). De conclusie was dat de meest stabiele resultaten werden verkregen met een methode die 'simplified reference tissue model' wordt genoemd, afgekort SRTM. Deze methode heeft in principe het voordeel dat het afnemen van bloed uit de polsslagader niet noodzakelijk is. Hierdoor is het scannen minder belastend voor vrijwilligers. Gebruik makend van deze methode, lieten de test-retest resultaten goede variabiliteit zien (14,0 ± 10,7%) en uitstekende betrouwbaarheid (ICC intraclass correlatiecoëfficiënt = 0,93) voor SRTM afgeleide BP_{ND}, een maat voor binding van de ligand aan de receptor. De ratio van striatum en cerebellum SUV minus 1, een benadering van BP_{ND}, bleek een uitstekende variabiliteit (6,2 ± 3,1%) met een uitstekende betrouwbaarheid (0,98) te laten zien en correleerde goed met SRTM (r^2 = 0,96), met een acceptabele (15%) bias. Tot slot, SRTM bleek het optimale model om [^{11}C]R116301 binding te kwantificeren, terwijl semi-kwantitatieve methoden zoals SUV ratios gebruikt zouden kunnen worden voor standaard klinische toepassingen in de toekomst.

Conclusie

De belangrijkste resultaten van dit proefschrift waren dat genetische- en omgevingsrisicofactoren voor angst en depressie van invloed kunnen zijn op gedeeltelijk verschillende hersenstructuren (amygdala en hippocampus), maar dat deze risicofactoren ook kunnen convergeren op dezelfde hersenstructuur (frontaal kwab). Daarom zou toekomstig beeldvormend onderzoek in de hersenen bij angst en depressie moeten worden uitgevoerd bij vrijwilligers die geselecteerd zijn op een ofwel genetisch ofwel omgevings bepaalde risicofactor.

Toekomstige studies met een zelfde design zouden functionele, structurele MRI en moleculaire neuro-imaging kunnen toepassen op andere neuropsychiatrische stoornissen die ook gebaseerd zijn op een gecombineerde genetische en omgevings gedreven etiologie.

APPENDICES

Dissertation series

Department of Psychiatry, VU University Medical Centre

N.M. Batelaan (2010). Panic an Public Health, Diagnosis, Prognosis and Consequences.
ISBN: 978 90 8659 411 5

G.E. Anholt (2010). Obsessive-Compulsive Disorder: Spectrum Theory and Issues in Measurement.
ISBN: 978 90 2345 442 7

N. Vogelzangs (2010). Depression & Metabolic Syndrome.
ISBN: 978 90 8659 447 4

C.M.M. Licht (2010). Autonomic Nervous System Functioning in Major Depression and Anxiety Disorders.
ISBN: 978 90 8659 487 0

S.A. Vreeburg (2010). Hypothalamic-Pituitary-Adrenal Axis Activity in Depressive and Anxiety Disorders.
ISBN: 978 90 8659 4917

S.N.T.M. Schouws (2011). Cognitive Impairment in Older Persons with Bipolar Disorder.
ISBN: 978 90 9025 904 8

P.L. Remijnse (2011). Cognitive Flexibility in Obsessive-Compulsive Disorder and Major Depression – Functional Neuroimaging Studies on Reversal Learning and Task Switching.
ISBN: 978 90 6464 449 8

S.P.A. Wolfensberger (2011). Functional, Structural, and Molecular Imaging of the Risk for Anxiety and Depression.
ISBN: 978 90 86595 36 5

Dankwoord

Vier kapiteins op een klein scheepje.

Allereerst wil ik graag alle gezonde proefpersonen en patiënten bedanken voor hun deelname aan het onderzoek, en voor hun geduld en vertrouwen in deze projecten. De tweelingen wil ik graag speciaal bedanken voor de lange scandagen die plaatsvonden in het weekend. Ik heb met veel plezier met jullie gewerkt.

Naast mijn mede-auteurs bedank ik graag iedereen die mij de afgelopen jaren heeft geholpen met het onderzoek. Een paar personen zou ik graag in het bijzonder noemen:

De promotiecommissie werd pas aan het einde van mijn promotie-periode definitief vastgesteld, waarin ieders kwaliteiten elkaar goed aanvulden.

Beste Witte, jij hebt me de mogelijkheid gegeven om te promoveren. Tot het einde van mijn promotietijd had jij de hoop dat de productie van [^{18}F]MPPF (humaan) alsnog op tijd werd goedgekeurd. Het humane MPPF protocol werd herschreven en goedgekeurd (warm en koud MPPF) in verband met veroudering van het eerste protocol. De rattenstudie met de $5HT_{1A}$ receptor ligand [^{18}F]MPPF werd wel uitgevoerd, maar kon in verband met praktische problemen moeilijk worden opgeschreven. In de tussen tijd zijn we, op initiatief van Adriaan, overgestapt op een studie met de [^{11}C]R116301 tracer welke eerder door onze groep werd toegepast op apen. En verder ben ik, door jou toedoen, de functionele en structurele MRI studie met de discordante twins met hoog en laag risico voor angst en depressie gaan doen. Een beter zij-traject had ik me niet kunnen voorstellen. Dank voor de geboden mogelijkheid.

Beste Adriaan, bij aanvang van dit project was jij de enige die als hoogleraar direct betrokken was bij dit project. Witte, Eco en Dick volgden je. Jij had de rust en de klasse om te bemoedigen waar dat nodig was, ongeacht de omstandigheden, en je verstaat je vak als de beste. Misschien was je net zo bevlogen geweest als je andere keuzes had gemaakt en voor de psychiatrie had gekozen ;-). Met name door jouw steun kreeg ik ook de mogelijkheid om kennis en ervaring op te doen buiten de Nederlandse landsgrenzen. Hopelijk volgen er in de toekomst meer psychiatrie/genetica studies op de afdeling nucleaire geneeskunde en PET research.

Beste Eco, als geen ander had jij oog voor het belang van het afronden van projecten. Met jouw ongeëvenaarde tempo en gedrevenheid is het nu af. Jij hebt je niet alleen gebogen over het twin design, maar vervolgens je vleugels over het gehele project uitgeslagen. Hoewel enigszins op afstand, heb ik juist heel erg veel aan je steun gehad. Je bijdrage was zeer verfrissend.

Beste Dick, jij hebt me intensief begeleid met de analyses van de MRI studies. Het verkopen van de oogst was een ingewikkelde klus, waarbij de prille eerste samenwerking vanuit de afdeling Psychiatrie met de afdeling Biologische Psychologie verder vorm kreeg.

De medewerkers van de afdeling Nucleaire Geneeskunde en PET Research en van de afdeling Radiologie wil ik graag bedanken voor de prettige en leerzame samenwerking. Jullie maakten me wegwijs in de wereld van de PET en de MRI. Ook de andere Neuro-imaging promovendi en post-docs: bedankt voor alle hulp en gezelligheid. In het bijzonder wil ik noemen de Michiel de Ruiter, Joost Kuijer en Marjan Nielen voor de hulp met de Sonata scanner en het in elkaar zetten van de taakjes en Bert Windhorst voor de adviezen van allerlei aard. Daarnaast speciale dank aan Amanda, Bart, Ronald, Mark, Maqsood, Robert, Gert, Henri, Patricia, Femke, Harry, Ab, Emile, Remi, Kevin, Carla en Virginie. De collega's op de J-vleugel, o.a Reina, Nikie, Jurgen, Floris, en Anke. En dan de dames van de neuroPET, Nelleke, Hedy, Ursula, en Alie: heel veel dank voor alle steun en gezelligheid. I would like to thank Kaoru Maruyama from Japan for the contribution to our work. It was an intensive and fruitful collaboration.

Verder wil ik de medewerkers van de afdeling Biologische Psychologie bedanken, in het bijzonder Dorret Boomsma, voor de steun en het vertrouwen en de geboden kans en Kim en Marcel voor hun betrokkenheid bij de twin studie. Zonder jullie was het niet gelukt.

Natuurlijk ook veel dank voor de hulp van de medewerkers van de afeling Psychiatrie, en met name de promovendi van de Psychiatrie/Neuro-imaging: Ursula, Peter en Odile: gedeelde smart is halve smart.

Verder ook dank aan alle medewerkers van de afdeling Klinische Genetica van het LUMC. Mijn naaste collega's, veel dank voor de gezelligheid aan Emmelien, Marije, Maartje, Dietje, en Gijs. In het bijzonder dank aan mijn opleider, professor Martijn Breuning, die mij uit het promotiemoeras –zo voelde het soms- heeft getrokken door mij de mogelijkheid te bieden de opleiding tot klinisch geneticus te volgen en mij te stimuleren dit boekje echt af te ronden. Een betere werkplek en een beter opleidingsklimaat kan ik me niet voorstellen. Heel hartelijk dank.

De leden van de leescommissie: prof.dr. K.L. Leenders, prof.dr. H.E. Hulshoff, prof.dr. F. Barkhof, prof.dr. R. Van Dyck. Allen bedankt voor het bestuderen van mijn proefschrift. Furthermore, I would like to thank professor Vincent Cunningham for his interest in my thesis. It is a great honour that you are a member of my reading committee.

De laatste loodjes... Esther Beekman, met het fabriceren van dit mooie boekje in mijn kraamperiode heb jij je enorm bewezen, niet alleen qua lay out, maar ook qua communicatie en improvisatie. Dank daarvoor.

En dan mijn fantastische paranimfen. Lieve Alie, vanaf dag 1 hebben wij lief en leed gedeeld in J-089. Het was heerlijk om jou als kamergenoot te hebben. Jij was de bindende factor van ontzettend veel gezelligheid. Bedankt voor de mooie tijd en hopelijk hebben we met het afronden van onze proefschriften wat meer tijd voor koffie.
Lieve Afke, jij hebt samen met mij de switch gemaakt van Medische Biologie naar Geneeskunde. Hoewel jij ook lange tijd in de VS hebt gewoond in de afgelopen jaren, hebben wij toch altijd met name onze prive avonturen kunnen delen. Dank je wel daarvoor

Daarnaast heb ik de afgelopen tijd veel steun en plezier gehad van en met mijn familie en de familie van Ian uit Suriname, van studiegenoten uit Antwerpen, van de medische biologie en geneeskunde. Mijn oude trouwe vrienden van het HML, Julia, Eveline, Rachel en Esther: bedankt voor alle warmte. De vrienden uit mijn Engeland periode, en andere vrienden: dank je wel voor heerlijke avondjes en telefoontjes. De buren, de ouders en juffen van de St. Jozefschool, in het bijzonder Ariaen, Roeselien, Katinka, en juf Reina, inmiddels directeur van de school, en de andere juffen: bedankt voor jullie gezelligheid en steun! Ingrid, bedankt voor met name alle telefoontjes: alles komt op z'n pootjes terecht. Ook speciale dank aan Lucia, voor de ondersteuning van binnen uit: je hebt er inmiddels je beroep van gemaakt.

Lieve papa en mama, dank voor alle stimulans en de enorme steun, niet alleen aan mij, maar aan alle gezinsleden. Ontzettend bedankt daarvoor. Mijn kinderen zullen jullie liefde dragen en doorgeven.

Lieve Lucas, Juliette en Samuel, bedankt voor jullie komst in ons leven, jullie vrolijkheid, onbezorgdheid, wijsheid en liefde. Jullie hebben mij de energie gegeven voor het bereiken van deze mijlpaal, en gezorgd dat ik te allen tijde alles kan relativeren.

Lieve lieve Ian, jij bent naar Nederland gekomen, patholoog geworden, wij zijn getrouwd en hebben drie kinderen gekregen en nog veel meer... allemaal tijdens mijn promotieperiode. Ik had je al met al toch een relaxtere start toegewenst in dit koude landje. Dank je wel voor je onvoorwaardelijke steun, je vertrouwen en je liefde voor de kinderen en mij. Jij bent mijn eeuwige stimulans om het allerbeste uit mezelf te halen en door te zetten. Dank je wel daarvoor en dank je voor nog ontzettend veel meer. Laten we ons 5 jaar huwelijk maar voor dit jaar op de agenda zetten (6 jaar dus ;-), een paar dagen na 11 mei...

About the author

About the author

Saskia Wolfensberger was born on June 26th, 1974 in Amsterdam, the Netherlands. At the age of three, she moved to Voorburg, and attended secondary school at the Montessori Lyceum in The Hague. In 1992-1993 she worked and studied English and Tourism at St. Clare's in Oxford and Christ Church College in Canterbury, UK, and went to Antwerp, Belgium to study Medicine in 1993-1994. She returned in 1994 to the Netherlands to study Medical Biology and Medicine at VU University Medical Centre, Amsterdam and received her Master's degree in Medical Biology in 1999, her Master's degree in Medicine in 2000, and after an internship in Suriname, she was registered as a general physician in 2002. She commenced her PhD training at the VU University Medical Centre in 2003, working at the departments of Psychiatry, Nuclear Medicine & PET Research, Radiology, and Biological Psychology. In 2009 she worked as a junior resident in the Geriatric Psychiatry clinic, Zuiderpoort, Haarlem and since 2010 she started her training as a resident at the department of Clinical Genetics at Leiden University Medical Centre. Saskia Wolfensberger is married to Ian Ambrose and is the mother of Lucas (2005), Juliette (2008) and Samuel (2010).