Supplemental Material

Methods

Recruitment

All subjects were given a Structured Clinical Interview (SCID) for DSM-IV to determine the presence of any psychiatric illness, a neurological examination, a battery of neuropsychological tests, an EEG, and a screening MRI examination. Exclusion criteria included inability to give informed consent, IQ below 70, significant medical problems, history of loss of consciousness for greater than five minutes, alcohol or drug abuse within the last 12 months, any electroconvulsive therapy within six months preceding recruitment, a history of significant neurological illness, and any focal abnormalities found by EEG or MRI.

fMRI Acquisition and Analysis

BOLD fMRI was performed on a GE Signa 3T (Milwaukee, WI) using gradient echo EPI (24 axial slices, 6 mm thickness, TR/TE = 2000/30 msec, field of view (FOV) = 24 cm, matrix = 64 x 64). Images were processed as described previously using SPM99 (http://www.fil.ion.ucl.ac.uk/spm). Images were realigned, spatially normalized into a standard stereotactic space (MNI template), smoothed with an 8mm full-width at half-maximum (FWHM)Gaussian filter and ratio normalized to the whole-brain global mean. First level intrasubject models were estimated using SPM99, where the two experimental conditions (0-back and 2-back) were represented by boxcars convolved with a canonical hemodynamic response.

Structural Image Processing

Three-dimensional volumes of T1-weighted SPGR images were acquired on a 1.5T GE scanner (General Electric, Milwaukee, WI) with a voxel resolution of 0.975 x 0.975 x 1.5 mm^3 (TR/TE/NEX 24/5/1; flip angle 45º; matrix size 256 x 256; FOV 24 x 24 cm; 124 sagittal slices). Data were analyzed on a Linux workstation (Red Hat 8.0) using MATLAB6.51SP1 (Mathworks, Natick, MA) and SPM2 (http://www.fil.ion.ucl.ac.uk/spm/; Wellcome Department of Imaging Neuroscience, London, UK). Additional imaging software packages used were Analysis of Functional NeuroImages (AFNI) (Cox, 1996), and Surface Mapping with AFNI (SUMA) (Argall et al., 2006).

Customized template creation was based on a sample of 100 healthy volunteers taken from our study sample. These subjects were grouped to best represent the sample as a whole, and were matched for age (NC mean = 35.6 (± standard deviation 9.97). The iterative procedure used to make the customized template optimally suited for our sample has been described previously (Pezawas et al., 2004).

Structural imaging data were preprocessed using AFNI and SPM2. We manually selected the permanent origin at the AC-PC line location. This was followed by optimized VBM performed in SPM2 using the non-uniformity correction option to minimize image inhomogeneities (Ashburner and Friston, 2000; Good et al., 2001). To correct for volume changes arising during normalization to the group template (described above), images were modulated by multiplication with the Jacobian determinant of the spatial normalization function. An integration of modulated gray matter voxel values as well as total white mater voxel values resulted in estimates for total gray matter and total white matter volume. The normalized and segmented images were smoothed using a 10mm (FWHM) kernel.

The normalized, modulated and smoothed gray and white matter images were analyzed using the General Linear Model as implemented in SPM2 (Friston, Holmes et al. 1995). We used a multiple regression model with RGS4 genotype, age, gender, total gray matter volume, WAIS-IQ and second-order polynomial age expansions as regressors in the

model.

Functional Connectivity

This measure examines the co-variation across the brain with the activation in a region (volume) of interest, using masks derived from functional clusters identified by the RGS4 rs951436 genotype correlation maps. These functional clusters became our seed volumes for subsequent functional connectivity analyses. After mean and drift correction of the time series, median activity within this region of interest was calculated for each scan (we prefer median as a robust estimator that coincides with the mean under the assumption of normality) and then correlated across the brain with all voxel time series, resulting in a map which contained, in each voxel, the correlation coefficient of the time series in this voxel with that of the reference regions. These maps (one per subject) were then analyzed in a random effects model in SPM identical to the one described in the previous paragraph.

Structural Covariance

Structural covariance reflects a similar measure of coupling as functional connectivity, only in this instance not between functional data (fMRI time series), but using voxel-wise gray matter volume maps derived from VBM (see above). Voxels identified as significant in the second-level VBM analysis of gray matter volume were used as seed volumes for subsequent structural connectivity analyses. This approach identifies regions that are positively or negatively correlated with the target area across subjects. The volume of an anatomically defined region of interest in standardized space was computed by adding the local volumes of all voxels comprising the ROI and used as a covariate of interest in a random-effects general linear model in SPM that also included nuisance covariates as described above for VBM. Statistical inference proceeded exactly as described for VBM

and functional connectivity by using appropriate contrasts to derive statistical maps for the significance of the volume covariate (to identify regions positively or negatively covarying in volume with the target region) or difference in correlation between the volume covariate split between genotype groups (to identify regions differing in their dependence on target area volume by genotype), achieving multiple comparison correction with the Gaussian random fields approach and restricting inference to appropriate regions of interest as detailed in the manuscript.

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