Supplementary Materials for:
DISC1 and SLC12A2 Interaction Affects Human Hippocampal Function and Connectivity

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Supplementary Methods:

We gathered BOLD fMRI data on a 3T scanner (GE Systems, Milwaukee, WI) using a GE-EPI pulse sequence acquisition across whole brain with 24 axial slices (TE=30 ms, TR= 2 seconds, flip angle=90°, field of view=24 cm, matrix=64 x 64, voxel dimensions=3.75x3.75x4 mm with a 1mm gap) (1). All subjects were healthy volunteers, both discovery (n=229) and replication (n=120) that gave written, informed consent to participate in the Clinical Brain Disorders Branch "Sibling Study" (NCT00001486, DR Weinberger, PI) (2). Our recruitment, screening, and inclusion criteria have been reported elsewhere (1-3). Briefly, in both cohorts, subjects were only included if they passed a rigorous medical and neurological exam (including blood work, neurological exam, and structural MRI), had an adequate IQ for consent (>70), a negative history for psychiatric illness individually or in first-degree relatives (obviously, not true for unaffected siblings), freedom from any psychotropic medications, and availability of high quality genetic and imaging data.

Our recognition memory task (3) presented visual stimuli consisting of an equal number of "indoor" or "outdoor" scenes with neutral emotional valence selected from the International Affected Picture System (4) randomly ordered throughout. The task evokes BOLD activation in both hippocampus and parahippocampal gyrus and a wider network shown to be involved in encoding and retrieval, with connectivity particularly directed to ventrolateral prefrontal cortex (VLPFC) (2-3,5). Visual instructions appeared briefly prior to each block. Four blocks of either encoding or retrieval (3 sec/stimulus; each conditions x 4 blocks x 20 sec/block) were interleaved with a simple cross-hair fixation rest condition. Each scene was novel during encoding, but during retrieval half were novel ("new") while half had been seen during encoding ("old").

Subjects were instructed to respond via a button press for "indoor" or "outdoor" during encoding and "new" or "old" during recognition (3).

We pre-processed and spatially normalized the fMRI data to the MNI common stereotaxic space using SPM5 (http://www.fil.ion.ucl.ac.uk/spm) followed by rigorous quality control, including for motion artifact (2). We entered single-subject contrast images (i.e., encoding vs. visual fixation) in a second level analysis treating subject as a random factor. The gene-by-gene interaction was modeled as a full factorial ANCOVA covaried for age and sex, with significance at $P< 0.05$ corrected for false-discovery rate (FDR-SVC) (6-7). To measure connectivity between hippocampus and VLPFC based on prior findings (5), we used psychophysiological interaction (PPI) (8). PPI maps the extent that hippocampal activity during recognition memory modulates regional activity elsewhere as dictated by the task design. We used a first level general linear model with regressors to deconvolve the fMRI signal from a seed within left hippocampus proper (the 'seed'), the task design, and the interaction between these two. Resultant PPI contrasts were entered into a full factorial ANCOVA just as above covaried for age and sex with a statistical threshold of $P< 0.05$ FDR-SVC (6-7).

Given our prior hypotheses, we restricted our search to the bilateral hippocampal formation (hippocam pus plus parahippocampal gyrus) using a search volume made with the WFU PickAtlas (http://fmri.wfubmc.edu/software/PickAtlas). This same tool was used to create a VLPFC region of interest for the PPI connectivity analyses. For replication, we used the initial activation and connectivity results (separately) as ROIs for activation and connectivity separately using MarsBaR (http://marsbar.sourceforge.net/index.html). It is important to note that both cohorts (discovery and replication) and both BOLD fMRI measures (activation and connectivity) were analyzed at the second level using separate, but identical random effects ANCOVA models

with identical covariates and contrasts. While our results are based on ANCOVAs using age and sex as covariates of no interest, we repeated all analyses (in both cohorts) without these covariates and found no significant differences in any result. Given the smaller sample size for replication, we calculated effect sizes (Cohen's d) from our initial results using VBM5 within SPM5 (http://dbm.neuro.uni-jena.de/vbm/). All figures were made using MRIcron (http://www.cabiatl.com/mricro).

Supplementary References:

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Supplementary Table S1: Demographics

Cohort	Genotypes	n	Age (years) mean (±SD)	Sex (M:F)	Education (years) mean (±SD)
Discovery		229			
	DISC1 GG-SLC12A2 CC	71	29.03 (8.07)*	35:36	16.61 ± 2.15
	DISC1 GG-SLC12A2 CT/TT	54	33.21 (9.94)	25:43	17.03 ± 2.42
	DISC1 GA/AA-SLC12A2 CC	68	31.69 (9.63)	27:27	16.93 ± 2.34
	DISC1 GA/AA -SLC12A2 CT/TT	36	29.26 (8.56)*	19:17	16.83 ± 2.29
Replication		120			
	DISC1 GG-SLC12A2 CC	49	33.6 (10.9)	21:28	16.4 (1.8)
	DISC1 GG-SLC12A2 CT/TT	23	32.9 (9.4)	9:14	17.3 (2.6)
	DISC1 GA/AA-SLC12A2 CC	33	32.5 (9.0)	10:23	16.4 (2.0)
	DISC1 AG/AA -SLC12A2 CT/TT	15	31.4 (10.7)	9:6	15.9 (2.6)

Table legend: Demographic characteristics for each cohort presented for each of four DISC1-SLC12A2 genotype subgroups. The only significant demographic difference was found in the discovery sample where DISC1 GG-SLC12A2 CC and DISC1 GA/AA -SLC12A2 CT/TT groups were younger than the DISC1 GG-SLC12A2 CT/TT group *($p < 0.05$). Age was used as a covariate in all subsequent analyses.