Supplementary Online content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1: Supplementary information about measures

Measure of stressful life events

Listed below are the 37 stressful life events included in the assessment of early adversity in the current study. Questionnaires were completed by the mother and collected at six time points after the birth of the child. The questionnaire when the child was 8 months old referred to events since the child was born. The questionnaires collected when the child was 21, 33, 47, 61, and 73 months old referred back to events that had happened within the last year.

Prenatal and adolescent adversity measures were used as control variables. The prenatal questionnaire at 18 weeks gestation referred to events since the mother became pregnant, and did not include questions 33 and 34. Adolescent adversity was measured on a single questionnaire at age 16 years, which referred to experiences while the participant was between 12 and 16 years old.

List of adversities assessed:

1. Mother's partner died
2. A sibling died
3. A family friend died
4. A sibling was ill
5. Mother's partner ill
6. Mother's friend ill
7. Mother admitted to hospital
8. Mother in trouble with the law
9. Mother divorced from her partner
10. Mother found out that the child's father did not want the child
11. Mother was very ill
12. Mother's partner lost job
13. Mother's partner had problems with work
14. Mother had problems with work
15. Mother lost job
16. Mother's partner left her
17. Mother's partner was in trouble with the law
18. Mother was separated from her partner
19. Mother's income reduced
20. Mother had a big argument with her partner
21. Mother had a big argument with family or close friends
22. The child moved to a new house
23. Mother's partner cruel to her
24. The family became homeless
25. Mother had financial problems
26. Mother's partner was physically cruel to the child or the child's sibling
27. Mother attempted suicide
28. Mother convicted of an offence
29. Mother started a new job
30. Mother had an abortion
31. Mother's partner was emotionally cruel to the mother
32. Mother's partner was emotionally cruel to the child or the child's sibling
33. Mother was physically cruel to the child or the child's sibling
34. Mother was emotionally cruel to the child or the child's sibling
35. Mother had an accident
36. Mother was under great stress due to an academic examination
37. The family's house was burgled

Measure of internalizing symptom

Pre- and early pubertal levels of internalizing symptoms (depressive/anxiety symptoms) were assessed using the Development and Wellbeing Assessment [DAWBA]. Measures were collected via maternal reports when the boys were 7, 10, and 13 years old. Questions followed the diagnostic criteria in the Diagnostic and Statistical Manual of Mental Disorders [DSM-IV] and the International Classification of Diseases [ICD-10]. We used ordered DAWBA categories that indicate the probability that a child will develop an internalizing disorder on a six-level scale ranging from very unlikely [0.1% of these children have an internalizing disorder] to probable [70% of these children have an internalizing disorder].

eAppendix 2: Supplementary information about the Activation Likelihood Estimation (ALE) definition of regions of interest

Activation Likelihood Estimation **(**ALE) is a meta-analytic technique used in the present study as a quantitative way of identifying brain regions that differ in glucose metabolism between patients with depression and healthy controls in a consistent way across multiple studies. The ALE technique was developed originally for meta-analyses of task-related functional neuroimaging (hence "activation" likelihood estimation) but can be applied in the same manner for results obtained with other imaging modalities. The ALE technique assesses the overlap of regions where differences in, for instance, metabolism, have been identified in different studies, by modeling each finding as a probability distribution centered on the reported coordinates.[1] We used results from previously published ^{18}F-fluorodeoxyglucose Positron Emission Tomography (^{18}FDG-PET) studies to create a quantitative map of brain regions where lower resting state glucose metabolism has been detected in depressed patients compared with healthy control participants. We used a location-based statistical approach rather than an effect-size based meta-analysis, given that we were more concerned with location than with magnitude of effects. Moreover, the use of coordinates for the ALE-based regions, rather than anatomical labels, minimizes the problem of mislabeling regions due to differences in anatomical referencing between studies.[2] A similar approach was taken by Sacher et al.,[3] yet this previous ALE included just 4 studies, whereas we identified 13 studies (see below for details of the literature search).

Literature search

To conduct the ALE we used existing literature that has identified regions with lower glucose metabolism in depressed patients relative to controls. Such studies were identified via a literature search on PubMed in January 2013. The search combined two statements (#1: depression [mesh] OR depression [ti] OR depression [ab]) and (#2: FDG-PET OR fluorodeoxyglucose). "Mesh" refers to the medical subject headings, "ti" refers to title and "ab" refers to abstracts containing the keywords entered. No time span was specified for date of publication. This search resulted in 842 initial papers. These papers were then examined to ensure the included studies met the following criteria: 1) the study used the ^{18}F-FDG-PET methodology; 2) the patient population met DSM-IV based diagnostic criteria for depression (bipolar or major depression); 3) the study included control participants; 4) glucose metabolism was measured at rest; 5) the study was not limited to a few preselected ROIs. We did not exclude patients based on medication as this would have seriously limited the number of studies and because the inclusion of medicated patients is commonly accepted in neuroimaging research.[4] Furthermore, a recent PET study found that lower metabolism observed in cortical regions was indeed more related to disease or mood states than to medication effects.[5]

Fifteen studies met the inclusion criteria for the current ALE[6-18]. Three of these studies[11,12,18] appeared to have run the relevant analyses, but did not provide the coordinates needed to run the ALE. These authors were contacted and two provided the necessary data upon request.[12,18] This resulted in inclusion of 14 studies and a sample size of 271 depressive patients and 193 controls in the ALE. eTable 1 summarizes information about the participants of the 14 studies included in the ALE dataset.[6-10,-12-18]

Subdivision of regions obtained from the ALE analysis

The ALE analysis resulted in a probability map consisting 17 clusters. Large clusters resulting from overlapping peaks/local maxima were further subdivided into smaller clusters with reference to the Automatic Anatomical Labeling atlas[19] using the PickAtlas software[20] in SPM8 (Wellcome Department of Imaging Neuroscience, London, UK) implemented in MATLAB 7 (Mathworks Inc., Sherborn, MA). We also split medial ROIs that projected across both hemispheres, into one ROI per hemisphere as FreeSurfer analyses each hemisphere separately. These divisions resulted in 36 clusters/ROIs of which 30 were classified as cortical and 6 were classified as subcortical. This paper focused only on the 30 cortical ROIs, which are shown in eFigure 1.

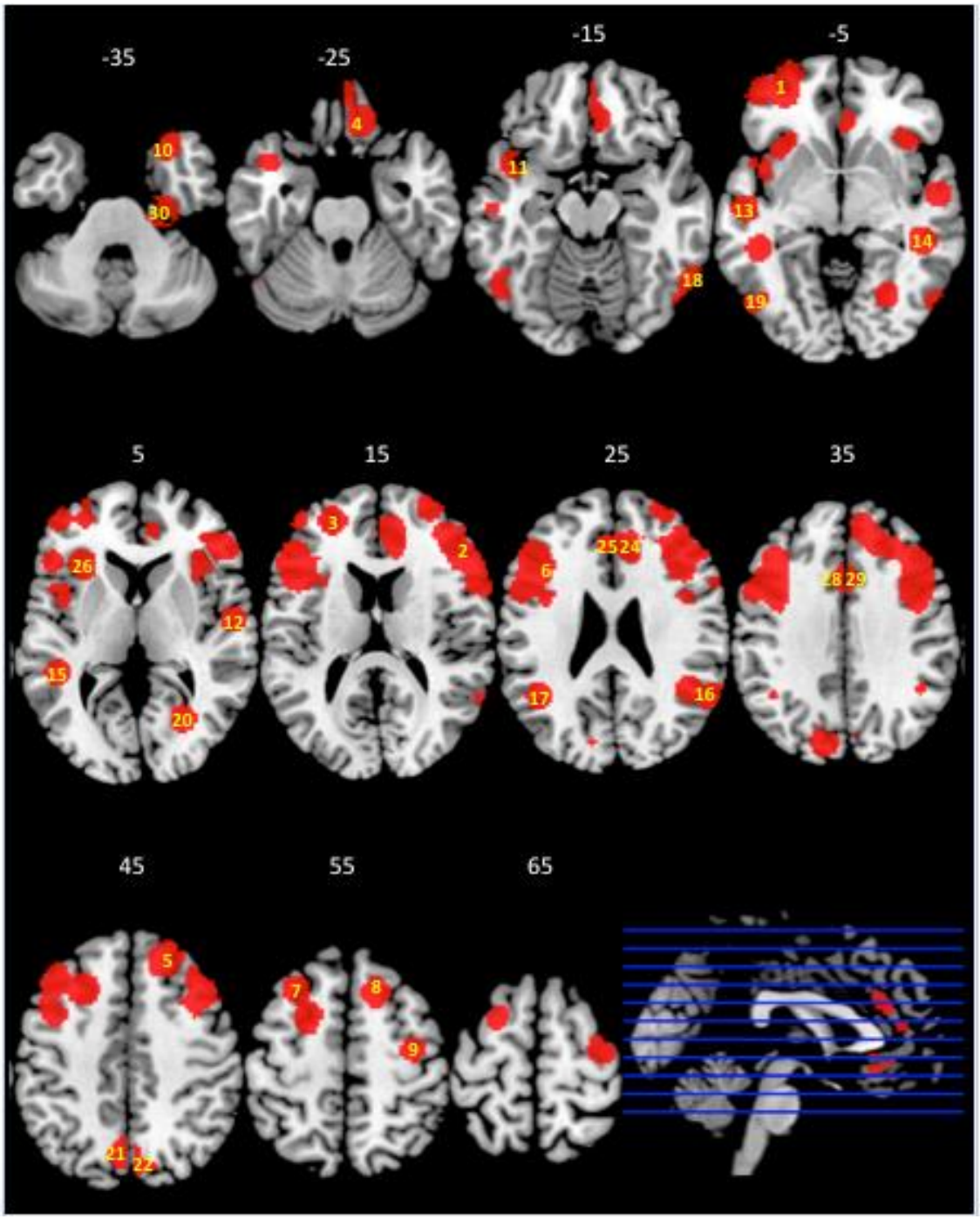
eTable 1. Information about the participant samples of the studies included in the ALE.

Authors	N Patients	N Controls	Diagnosis	Age (Mean±SD)		Gender ratio (Male/Female)		Pharmaco-therapy?
				Patients	Controls	Patients	Controls	
Broody et al., 2001	24	16	MDD	38.9±11.4	35.6±18.3	13/11	8/8	No
Brooks et al., 2009a	16	11	Bipolar	58.7±7.5	58.3±5.2	14/2	10/1	Yes
Brooks et al., 2009b	15	19	Bipolar	36.1±10.4	34.0±13.3	7/8	12/7	No
Brooks et al., 2010	8	8	Bipolar	52.8±8.5	55.9[a]	7/1	7/1	Yes
Drevets et al., 1997	17	12	MDD	35±9.4	34±8.2	10/17	11/21	Yes
Hosokawa et al., 2009	47	20	MDD / Bipolar	48.2±16.6	47.6±15.1	0/47	0/20	Mixed
Kegeles et al, 2003	19	10	MDD / Bipolar	36.0±11.0	39.0±19.0	6/13	4/6	Yes
Kennedy et al, 2001	13	24	MDD	36.76±9.37	31.7±6.7	10/0	24/0	No
Martinot et al., 2011	31	39	MDD	47.7±7.3	45.2±11.8	11/20	14/25	Mixed
Mayberg et al., 2001	18	13	MDD	45.0±12.0	38.0±11.0	15/3	12/3	Yes
Yuuki et al., 2005	7	10	MDD	58.0±9.4	60.8±7.9	4/3	5/5	Yes
Suwa et al., 2012	16	11	MDD	32.8±7.9	38.9±10.9	10/6	7/4	Yes
Zhang et al., 2011	40	20	Bipolar	43.4±2.7	N/A	16/24	N/A	Yes
Total	**271**	**193**						

N = population size; MDD = Major Depressive Disorder; Bipolar = Bipolar Disorder; SD = Standard Deviation.
Notes: [a]: SD was not available

eFigure 1. Regions of interest (ROIs) identified in the ALE

Image showing the 30 cortical regions of interest (ROIs) identified from the ALE analysis and separated in smaller regions using PickAtlas. The ROIs are shown on transverse slices ranging from z=-35 to z=65 (Montreal Neurological Institute coordinates). Yellow numbers refer to the ROI numbers presented in Table 1 in the main manuscript and eTable 2.



eAppendix 3: Supplementary information about the extraction of measures of cortical grey matter (GM) volume, cortical thickness and surface area in FreeSurfer

For every MR image, FreeSurfer segments the cerebral cortex, the white matter, and other subcortical structures, and computes meshes with ≈160,000 triangles that recover the geometry and topology of the pial surface and the gray/white interface of the left and right hemispheres. Regional measures of GM volume, thickness and surface area were obtained by projecting each of the 30 ROIs onto the cortical surface generated using a series of FreeSurfer functions. First, the 30 cortical ROIs were projected from three-dimensional volumes onto the cortical surface of the average brain using *mri_vol2surf*. The 30 surface ROIs were bound into "annotation" files for the left and right hemisphere and then projected from the average template into the participant's native space using *mri_surf2surf*. Cortical volume, surface area and thickness were then calculated from all ROIs for all participants using *mris_anatomical_stats*. Total brain volume is the total volume of all gray and white matter. Local cortical thickness is measured as a distance between the position of homologous vertices in the pial and gray/white surfaces. A correspondence between the cortical surfaces across participants is established using a nonlinear alignment of the principal sulci in each participant's brain with an average brain.[21]

eResults: Results from univariate and ultivariate models

ETable 2 shows the effects of early adversity and internalizing symptoms on cortical GM volume from the initial univariate models that were part of the model building strategy. Detailed results from the final multivariate path model, which included only the three regions that remained significant after the FDR correction, are found in eTable 3.

eTable 2. Results from the univariate models.

Effects of early adversity and internalizing symptoms on cortical GM volume. Significant associations ($p < .05$) are shown in bold, and their p-value is highlighted with bold. Control variables are not included in the table.

Risk factor	ROI number	β	SE	*P value*
L frontopolar (lateral)	1			
Early adversity		-.050	.059	.402
Internalizing symptoms		**-.120**	**.055**	**.029**
R mid-dorsolateral frontal cortex	2			
Early adversity		.003	.051	.951
Internalizing symptoms		-.054	.045	.230
L frontopolar (lateral)	3			
Early adversity		-.082	.064	.201
Internalizing symptoms		.036	.063	.566
R ventromedial prefrontal cortex	4			
Early adversity		-.037	.057	.510
Internalizing symptoms		-.030	.052	.569
R frontal medial cortex	5			
Early adversity		-.053	.070	.449
Internalizing symptoms		-.067	.056	.228
L mid-dorsolateral frontal cortex	6			
Early adversity		-.011	.058	.854
Internalizing symptoms		-.025	.048	.611
L superior frontal sulcus	7			
Early adversity		-.038	.061	.526
Internalizing symptoms		-.084	.058	.144
R superior frontal gyrus	8			
Early adversity		.040	.072	.577
Internalizing symptoms		**-.202**	**.067**	**.002**
R frontal precentral sulcus	9			
Early adversity		.017	.070	.810
Internalizing symptoms		-.032	.064	.614
R temporal pole	10			
Early adversity		-.048	.073	.512
Internalizing symptoms		.086	.068	.205
L temporal pole	11			
Early adversity		-.102	.075	.174
Internalizing symptoms		.061	.073	.406
R superior temporal gyrus	12			
Early adversity		-.069	.067	.303
Internalizing symptoms		.003	.064	.964
L superior temporal sulcus (anterior)	13			
Early adversity		-.129	.069	.061
Internalizing symptoms		.107	.055	.050
R superior temporal sulcus (posterior)	14			
Early adversity		-.123	.071	.083
Internalizing symptoms		.009	.060	.878
L superior temporal sulcus (posterior)	15			
Early adversity		-.045	.068	.507
Internalizing symptoms		.033	.061	.590
R supramarginal/angular gyrus	16			

Early adversity		**.159**	**.073**	**.029**
Internalizing symptoms		-.068	.064	.288
L supramarginal/angular gyrus	17			
Early adversity		.106	.077	.167
Internalizing symptoms		**-.117**	**.056**	**.036**
R fusiform gyrus	18			
Early adversity		-.075	.072	.297
Internalizing symptoms		-.075	.061	.220
L fusiform gyrus	19			
Early adversity		**-.136**	**.068**	**.045**
Internalizing symptoms		.020	.060	.736
R lingual gyrus	20			
Early adversity		.028	.065	.671
Internalizing symptoms		.107	.063	.091
L precuneus	21			
Early adversity		.022	.071	.751
Internalizing symptoms		.071	.062	.251
R precuneus	22			
Early adversity		**.173**	**.068**	**.011**
Internalizing symptoms		-.050	.066	.444
L parieto-occipital sulcus	23			
Early adversity		-.034	.081	.677
Internalizing symptoms		.038	.080	.637
R anterior cingulate sulcus (rostral)	24			
Early adversity		-.053	.062	.389
Internalizing symptoms		-.002	.049	.974
L anterior cingulate sulcus (rostral)	25			
Early adversity		-.064	.065	.328
Internalizing symptoms		-.104	.054	.055
L insula (anterior)	26			
Early adversity		.048	.057	.398
Internalizing symptoms		-.064	.052	.218
R insula (anterior)	27			
Early adversity		.065	.068	.332
Internalizing symptoms		-.040	.057	.479
L anterior cingulate sulcus (caudal)	28			
Early adversity		.127	.069	.067
Internalizing symptoms		-.081	.061	.180
R anterior cingulate sulcus (caudal)	29			
Early adversity		**-.173**	**.068**	**.011**
Internalizing symptoms		.083	.067	.210
R parahippocampal gyrus	30			
Early adversity		-.019	.064	.770
Internalizing symptoms		.049	.065	.450

Vol. = volume, B= standardized path coefficients, SE = Standard Error, p = probability value.

eTable 3. Results from the final multivariate path model.

Effects of early adversity and internalizing symptoms on cortical GM volume. Significant associations of interest are shown in bold, control variables are shown in italics.

Risk factor	**Mean volume [voxels]**	**SD volume**	**β**	**SE**	***P value***
Total brain volume	717985	52360			
2. R superior frontal gyrus	805	170			
Early Adversity			.047	.072	.520
Internalizing symptoms			**-.204**	**.067**	**.002**
Total brain vol.			*.338*	*.040*	*<.001*
Adolescent adversity (12-16y)			*.041*	*.048*	*.390*
Prenatal family stress			*.060*	*.057*	*.295*
Duration breast feeding			*-.042*	*.045*	*.348*
5. R precuneus	992	196			
Early Adversity			**.178**	**.068**	**.009**
Internalizing symptoms			-.054	.066	.410
Total brain volume			*.379*	*.039*	*<.001*
Adolescent adversity (12-16y)			*.019*	*.050*	*.701*
Prenatal family stress			*-.110*	*.057*	*.058*
Duration breast feeding			*.033*	*.045*	*.460*
6. R Anterior cingulate sulcus	642	180			
Early Adversity			**-.177**	**.068**	**.010**
Internalizing symptoms			.084	.067	.308
Total brain volume			*.266*	*.041*	*<.001*
Adolescent adversity (12-16y)			*.004*	*.054*	*.941*
Prenatal family stress			*.041*	*.061*	*.504*
Duration breast feeding			*-.081*	*.047*	*.082*

SD = Standard Deviation, B= standardized path coefficients, SE = Standard Error, P value = probability value.

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