Functional Brain Activation to Emotionally Valenced Faces in School Aged Children with a History of Preschool Onset Major Depression

Supplemental Information

Original Preschool Depression Study Sample Recruitment

Preschoolers between 3 and 6 years of age were recruited from sites throughout the Saint Louis area for participation in a study examining the nosology of preschool depression. Recruitment was done through primary care practices, and preschools/daycares that were accessible to the general community in order to enhance the socioeconomic and ethnic diversity of the sample. Recruitment sites were chosen at random using a geographically stratified method. The aim of this sampling technique was to recruit a large group of depressed preschoolers as well as smaller groups of disruptive and healthy preschoolers for comparison. To achieve this goal, a validated screening checklist, The Preschool Feelings Checklist (PFC) was completed by caregivers (1). Previous studies have indicated that a PFC score of 3 or greater had high sensitivity and specificity for the diagnosis of depression (1). In addition to identifying and including children with high symptom sum scores, children with low or no endorsed symptoms were also recruited in order to establish an adequate healthy comparison group. Approximately 6000 checklists were distributed to sites between May 2003 and March 2005. In daycares and preschools, from which approximately 3/4 of sample was ascertained, checklists were handed out to all parents of children in the target age range. Checklists were made available in waiting areas of primary care settings next to a poster describing a study of early emotion development. Completed checklists were collected by the sites and returned. Using this method, n = 1474 checklists were returned and those with scores of 0 (presumed healthy) or 3 or greater (above established cut-off) were contacted for further participation. Among those checklists returned, n = 335 were ineligible due to being out of the age range and n = 240 had PFC scores out of range (e.g., 1 or 2). The remaining n = 899 met all initial

screening and inclusion criteria and were contacted by phone for further screening. Based on phone screening, subjects with chronic illness, marked speech and language delays and/or neurologic or autistic spectrum disorders were excluded. Those without exclusions (*n* = 416) were invited for study participation and *n* = 305 agreed and presented for the assessment.

Comparability of Scanned Sample to Original Sample

The original 305 children included those recruited because they had preschool onset major depressive disorder (PO-MDD), were "healthy" controls, or had a disruptive or anxiety disorder. We started scanning after the 4th wave of annual assessments, and we were funded to recruit only those children for the scanning portion who had been diagnosed with an episode of PO-MDD or who had remained healthy. This provided an eligible pool of 96 children with PO-MDD and 86 healthy children to approach in regards to participation in the scanning portion of the study. Of these, 65 children (39 PO-MDD and 26 healthy) children had been scanned. Of these 65, 17 had data which had to be excluded because of incomplete runs or poor quality data (of note, this percentage is not unusual for child studies, but improved over start of the study as our methods for ensuring compliance and low movement improved). Of the 32 PO-MDD children with usable data, 10 were on medications at some point, and were thus excluded. We did several things to ensure that the children included in the current analyses were comparable to the full sample. First, the percentage of children on meds in the PO-MDD group with usable data was 31%. The percentage of children in the PO-MDD group with unusable data who were medicated was 42% (3/7). The percentage of children in the PO-MDD group who have not yet been scanned who were medicated (based on previous assessment data) was 39% (22/57). Thus, the percentage of PO-MDD children in the scanned sample was not meaningfully different than the not scanned sample. We then asked if the scanned and unscanned PO-MDD children differed on clinical variables at the baseline assessment, and they did not differ on depression scores ($p > .6$), externalizing scores ($p > .35$), or internalizing scores

(p > .34). They also did not differ on gender (60% male versus 51% male) or ethnicity (44% Caucasian versus 49%). They did not differ on parental education (p > .15). Thus, these data make us fairly confident that these children are relatively representative of the original sample.

Diagnostic Assessment

The children were between the ages of 3.0-5.11 at the time of their first interview and between the ages of 7.0-10.11 at the time of scanning. The first three interviews used the Preschool-Age Psychiatric Assessment (PAPA) (2) and were audiotaped and reviewed for reliability as previously reported (3). The PAPA is designed for diagnostic use with children ages 2.0-6.0 (but has been used up to age 8.0) years, has acceptable reliability (2), and consists of a series of developmentally appropriate questions answered by the primary caregiver which cover the DSM-IV criteria for all Axis I disorders, including MDD, attention-deficit/hyperactivity disorder (ADHD), and anxiety disorders. The two-week duration requirement for MDD was omitted based on prior data suggesting it is not a clinically sensitive cut-off in the preschool age group (4,5). At the most recent time point, the interview completed was the Childhood and Adolescent Psychiatric Assessment (CAPA) that allows for assessment of participants aged 9.0-17.11. The CAPA uses reports from the child or adolescent in addition to the primary caregiver (6). Combined parent and child reports using the methods described by Bird (7) were used at these assessment waves. Children were classified as having a history of PO-MDD if the child met symptom criteria for MDD on the PAPA prior to age 6.0 (5). For each in-person diagnostic time point, depression sum scores (the total number of MDD symptoms endorsed in the PAPA/CAPA) were calculated and used to assess symptom severity (8). We also computed summed severity scores for non-depression internalizing disorders (social anxiety disorder, generalized anxiety disorder, specific phobia, posttraumatic stress disorder) and externalizing disorders (oppositional defiant disorder, conduct disorder, ADHD) at each time point. We used these scores to examine whether results were specific to depression or were related more

generally to internalizing or externalizing psychopathology. Child and parent versions of the Children's Depression Inventory (CDI-C and CDI-P) were completed at the time of scan (9) to assess current MDD symptom severity.

Additional Functional Task and Stimuli

The task was programmed in PsyScope, and behavioral responses in the scanner were acquired via a fiber optic button box interfaced with the PsyScope button box. The images were projected onto a computer screen behind the subject's head within the imaging chamber. The screen was viewed by a mirror positioned approximately 8 cm above the subject's face.

Sad Mood Elaboration Procedures

The sequence of events for the sad mood induction is outlined below.

> Experimenter: "For the next minute, I just want you to lay still and look at the cross in the center of the screen." [Fixation period images collected (TR: 2.5, Frames: 25)]
>
> Experimenter: "How do you feel right now? Pick the face that best matches how you feel and tell me the number." Showing the child 5 schematic images of emotional faces (1 = very sad, 2 = somewhat sad, 3 = neutral, 4 = somewhat happy, 5 = very happy)
>
> Experimenter: "During this next scan, we are going to show you a film clip. After the film clip, you will hear some instructions. You will be asked to think about the situation and how you would feel."
>
> Sad clip from "*My Girl"* is played while T2 images are collected.
>
> Experimenter: "Think about the situation that you just watched…<Pause for 6 seconds>." "Imagine yourself being in this situation…<Pause for 21 seconds>." "Try to really concentrate on this feeling… <Pause for 20 seconds>." "Have you ever been in a similar situation? Have you ever lost a loved one and if so, how did it make you feel? We will now give you a minute to think about this while we do some scanning." [Elaboration period images collected (TR: 2.5, Frames: 25)]
>
> Experimenter: "How do you feel right now? Pick the face that best matches how you feel and tell me the number." Showing the child 5 schematic images of emotional faces (1 = very sad, 2 = somewhat sad, 3 = neutral, 4 = somewhat happy, 5 = very happy)

Functional Data Acquisition and Processing

For structural data, three-dimensional T1-weighted images (TR = 2,400 ms, TE = 3.16 ms, flip angle = 8°, slab = 176 mm, 176 slices, matrix size = 256 x 256, field of view (FOV) = 256 mm, voxel size = 1 x 1 x 1 mm, 1 signal average) were acquired in the sagittal plane with the use of MP-RAGE (magnetization-prepared rapid gradient echo) sequence. Blood oxygen level dependent (BOLD) images during face processing were acquired with a T2*-weighted asymmetric spin-echo echo-planar sequence (TR = 3000 ms, TE = 27 ms, flip angle = 90°, FOV = 256 mm, voxel size = 4 x 4 x 4 mm) in the axial plane paralleling to the anterior-posterior commissure, with a 12-channel head coil. During each functional run, 99 sets of 36 contiguous axial images with isotropic voxels (4 mm^3) were acquired parallel to the anterior-posterior commissure plane.

fMRI Preprocessing

The fMRI data were preprocessed using standard preprocessing steps, including: 1) Compensation for slice-dependent time shifts; 2) Removal of first 5 images of each run to allow BOLD signal to reach steady state; 3) Elimination of odd/even slice intensity differences due to interpolated acquisition; 4) Realignment of data acquired in each subject within and across runs to compensate for rigid body motion (10); 5) Intensity normalization to a whole brain mode value of 1,000; 6) Registration of the 3D structural volume (T1) to the atlas representative template in the Talairach coordinate system (11) using a 12-parameter affine transform and re-sampling to 1 mm cubic representation (10,12); and 7) Co-registration of the 3D fMRI volume to the T2, and the T2 to the participants structural image; 8) transformation of the fMRI volumes to atlas space using a single affine 12-parameter transform and 9) spatial smoothing using a 6 mm full-width half-maximum Gaussian filter. The common atlas template was optimized for children in our age range, and the use of co-registration to this type of common template space has been validated in several prior studies (13,14).

Analysis of fMRI data was performed using in-house software (FIDL analysis package, http://www.nil.wustl.edu/Bfidl) utilized in numerous previously published studies (15-20). Estimates of BOLD response to each face type within each subject were obtained using fixed effects general linear models (GLM) incorporating regressors for linear trend and baseline shifts. A hemodynamic response shape was assumed (Boynton function) and used to derive magnitude estimates relative to fixation baseline. These single subject estimates were then entered into group level analyses that treated subjects as random effects, as described in the main text. One subject was identified as a univariate outlier for one of the correlational results (though not a bivariate outlier). However, all results remained statistically significant if this subject was removed.

A Priori Region of Interest (ROI) Definition

The amygdala, hippocampal and striatal regions used anatomical templates (21-23). The dorsal lateral prefrontal cortex ROIs were defined on an atlas-representative image using the boundaries described by Rajkowska and Goldman-Rakic (24,25). The cingulate regions used centroids of activation identified in prior studies (26-28), around which we drew 25 mm diameter spherical ROIs edited to respect gray matter boundaries on an atlas-representative image.

Whole Brain Analyses Correlational Analyses

The voxel-wise correlations between baseline (Time 1) MDD severity and brain activation in response to sad versus neutral faces across the whole brain revealed positive correlations in a number regions (see Table S1), including orbital and superior frontal cortex, bilateral parietal cortex, medial and lateral temporal regions, parahippocampal cortex, occipital cortex and the cerebellum. As with the correlations in the a priori ROIs, greater baseline depression severity was associated with greater activation in response to sad versus neutral

faces in these regions. Again, all of these correlations remained significant when we included externalizing and non-MDD internalizing symptoms as covariates, as well as parent and child reported CDI scores at the time of scan. We then examined whether these associations were specific to activation to sad faces, or also present for activation to angry or fearful faces. As shown in Table S1, the superior and orbital frontal correlations were specific to sad faces, as were 3 out of the 4 parietal correlations. In the occipital cortex, the correlations in the more inferior regions were specific to sad faces, while the more superior regions and the lingual gyrus also showed correlations between baseline depression severity and either angry and/or fearful faces. There was more variability in the pattern of correlations shown in the temporal and parahippocampal regions. Among the parahippocampal regions, the right hemisphere regions showed correlations specific to sad faces, while the left hemisphere regions showed correlations between baseline depression severity and angry and/or fearful faces as well as sad faces. The majority of the other temporal cortex regions also showed correlations with fearful/angry faces as well as sad faces. In the cerebellum, the left hemisphere regions showed a correlation specific to sad faces, but the right hemisphere regions showed correlations with angry and/or fearful faces as well as sad faces. Thus, as with the a priori ROIs, the correlations were specific to sad faces in many, though not all, regions.

We also examined correlations between average depression severity across all assessment time points and activation to sad versus neutral faces, but like the analysis within the a priori ROI mask, the whole brain analysis did not identify any significant regions. In addition, we also examined whether baseline (Time 1) depression severity was correlated with activation to either angry or fearful faces versus neutral faces in voxel-wise whole-brain correlational analyses. These analyses identified a single region that showed a positive correlation between baseline depression severity and activation in angry faces relative to neutral faces, in left superior frontal cortex (X = -21, Y = 19, Z = 61). Thus, these analyses again

suggest a relatively stronger relationship between baseline depression severity and activation to sad faces versus angry or fearful faces.

Table S1. Correlations Between Baseline Depression Severity Scores and Functional Brain Activation in Whole Brain Analysis

Region	BA	X	Y	Z	ROI Z Value	Correlation with Sad vs. Neutral Faces	Correlation with Angry vs. Neutral Faces	Correlation with Fear vs. Neutral Faces
Frontal Cortex								
Left Orbital Frontal Cortex	13	-36	11	-09	3.95	**.60**	.11	.28
Left Superior Frontal Cortex	6	-24	2	55	3.81	**.59**	.33	.12
Parietal Cortex								
Right Inferior Parietal Lobule	40	32	-55	43	4.09	**.62**	.31	.32
Left Inferior Parietal Lobule	40	-38	-46	46	4.13	**.63**	.28	.28
Left Superior Parietal Cortex	3	-23	-31	48	3.81	**.59**	.09	-.01
Left Superior Parietal Cortex	7	-18	-58	49	4.13	**.62**	**.37***	**.42****
Occipital Cortex								
Right Cuneus	30	6	-64	9	4.00	**.61**	.24	.36
Right Lingual Gyrus	18	24	-74	-9	4.04	**.61**	.36	.35
Left Inferior Occipital Cortex	18	-36	-83	-4	3.85	**.59**	.23	.20
Right Occipital Cortex	19	45	-68	3	4.09	**.62**	.30	.35
Right Cuneus	19	29	-78	30	4.00	**.61**	**.37***	.18
Right Cuneus	18	5	-86	17	3.71	**.57**	**.37***	.33
Left Occipital Cortex	19	-29	-81	23	3.98	**.61**	**.44***	**.39***
Lingual Gyrus	18	0	-90	-02	3.75	**.58**	**.43****	**.48****

Temporal Cortex								
Right Parahippocampal Cortex	35	22	-46	8	4.2	**.63**	.23	.26
Right Parahippocampal Cortex	36	27	-34	-13	3.69	**.57**	.18	.27
Left Parahippocampal Cortex	35	-16	-22	-17	4.14	**.63**	**.38***	**.44****
Left Parahippocampal Cortex		-27	-57	3	3.73	**.58**	.32	**.47****
Left Lateral Temporal Cortex	21	-49	-18	-11	3.69	**.57**	.20	.28
Right Lateral Temporal Gyrus		45	-40	0	3.80	**.58**	**.45****	**.48****
Right Lateral Temporal Gyrus	20	47	-20	-18	3.94	**.60**	**45****	.32
Left Lateral Temporal Gyrus	37	-44	-43	-02	3.91	**.60**	.32	**.48****
Uncus	--	35	1	-23	4.32	**.65**	**.50****	.33
Cerebellum								
Left Cerebellum	--	-29	-53	-41	3.93	**.60**	.31	.31
Right Cerebellum	--	33	-53	-37	4.28	**.64**	**.54****	**.43****
Right Cerebellum	--	5	-36	-29	3.95	**.60**	**.39***	**.40***

BA, Brodmann area; ROI, region of interest.
Gray shading indicates regions that displayed significant correlations only with activation to sad versus neutral faces.
* $p < .05$
** $p < .005$

Supplemental References

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