NEURAL RESPONSE TO PEER REJECTION IN CLINICALLY DEPRESSED ADOLESCENTS AND HEALTHY CONTROLS

A Dissertation

Presented to

The Faculty of the Department of Psychology

University of Houston

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

By

William Mellick

May, 2017

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ABSTRACT

Major depression is a debilitating and highly recurrent mental illness that typically emerges during adolescence (Hankin, 2006). The increased vulnerability to depression observed during adolescence is posited to arise in part from major neurobiological changes that occur during typical development (Davey, Yücel, & Allen, 2008). Importantly, these neurobiological changes occur while the adolescent's focus shifts from parents to peers (Steinberg, 2005). Social reward (e.g., peer acceptance) is therefore thought to be particularly salient, and a failure to obtain social reward (e.g., through social rejection) has been implicated as a driving force in the vulnerability to and maintenance of adolescent depression (Davey et al., 2008; Mellick, Sharp, & Ernst, 2015). Indeed, social rejection during adolescence is highly predictive of depression (Prinstein & Aikens, 2004), and currently depressed adolescents experience more rejection than their healthy peers (Lee, Hankin, & Mermelstein, 2010). Several adult and youth depression studies have examined response to social rejection revealing greater distress among depressed individuals; however, these studies relied predominantly on self-report or behavioral data. Therefore, the neurobiological underpinnings of rejection in depression remain underexplored despite the fact that such findings may inform etiological and theoretical models of depression, helping to further classify depression in terms of neural circuitry.

Against this background, a total of $N = 35$ adolescents were recruited to form two groups (Depressed, $n = 17$; Healthy controls, $n = 18$) who experienced rejection during fMRI scanning. This study had two aims: 1) To compare neural response to peer rejection in depressed adolescents versus healthy controls, and 2) To examine sex as a moderator of the relation between depression and neural response to rejection. Whole-brain voxel-wise and region of interest (ROI) between-group analyses were performed. Whole-brain results showed depressed adolescents to exhibit significantly greater rejection response in the right anterior insula, left

occipital operculum, and left nucleus accumbens. Reduced ventral striatal response to social inclusion was not found in depressed adolescents. ROI analyses led to null findings with no significant differences observed between groups. Insufficient samples sizes prohibited examining sex as a moderator. Exploratory tests of pubertal x group effects were conducted though non-significant, which was presumably due to limited data. Positive and null results are discussed in relation to extant neuroimaging findings in healthy and depressed samples with an emphasis on discrepancies across studies which may be due to methodological differences. The present study was among the few to recently employ Cyberball in the study of psychiatric populations marked by interpersonal functioning deficits and contributes to the identification of unique and/or shared neural substrates of an important interpersonal process in adolescent depression.

NEURAL RESPONSE TO PEER REJECTION IN CLINICALLY DEPRESSED ADOLESCENTS AND HEALTHY CONTROLS

Background and significance

Marked by primary features of depressed mood and anhedonia, major depression is a debilitating mental illness associated with significant physical, emotional, and behavioral impairment in a range of contexts (i.e., family, school, and social; Goodyer & Sharp, 2005). Depression ranks as the fourth leading cause of disease burden worldwide (Üstün, Ayuso-Mateos, Chatterji, Mathers, & Murray, 2004), with cost estimates of $83.1 billion (Greenberg et al., 2003) and prevalence rates of 16.6% lifetime and 7.1% for 12-months, respectively (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). Adolescent depression, with an estimated prevalence rate of 11.2% (Merikangas et al., 2010), is of particular concern because major depression typically emerges during adolescence and 75% of adolescent cases are reported as severe (Hankin, 2006; Merikangas et al., 2010). Even at subclinical levels adolescent depressive symptoms are highly predictive of adult depression (Pine, Cohen, Cohen & Brook, 1999). It is therefore imperative that we improve our understanding of mechanisms involved in the development and maintenance of adolescent depression in an effort to improve early intervention and treatment methods.

Depression has long been conceptualized as a highly interpersonal disorder given that depression is associated with impaired interpersonal functioning which often results in social rejection (Coyne, 1976; Strack & Coyne, 1983; Joiner, Metalsky, Katz, & Beach, 1999). For instance, Coyne (1976) found depressed adults to elicit depression, anxiety, and hostility in healthy adults while being described as less pleasant, wishing to appear sad, low, passive and more uncomfortable during social interactions, resulting in a greater likelihood of rejection. Other adult studies have confirmed that this type of negative interpersonal style among depressed

individuals along with higher rates of rejection (Mullins, Peterson, Wonderlich, & Reaven, 1986; Strack & Coyne, 1983; Joiner et al., 1999). Given the nature of adolescence, interpersonal rejection may be particularly relevant to conceptualizing adolescent depression.

Adolescence is a period of heightened vulnerability to depression due to the dramatic biological, psychological, and social changes that occur during typical adolescent development (Davey et al., 2008). The reward system continues to develop and its connections with prefrontal regions become more finely-tuned, as only frequently used synapses are strengthened and maintained (Durston et al., 2006). This maturation is associated with an enhanced ability to encode more complex rewards which are often more salient and social in nature (Davey et al., 2008). This complexity is in part a function of the adolescents' increased capacity for abstract mental representation which occurs concomitantly with prefrontal development (Eccles, Wigfield, & Byrnes, 2003). These neurodevelopmental processes occur while the adolescent's social environment is also dramatically changing: the focus shifts from parents to peers (Steinberg, 2005), more time is spent with peers (Csikszentmihalyi & Larson, 1984), and peer acceptance is of great concern (Parkhurst & Hopmeyer, 1998). Thus, changes in the adolescents' social environment, in conjunction with typical psychological and biological development, are posited to make social reward (e.g., social acceptance) especially salient and motivating for adolescents (Davey et al., 2008). While social reward becomes more important, social rejection occurs at its highest rates during adolescence (Juvonen, Graham, & Schuster, 2003). Moreover, social rejection in one domain (e.g., peers) is likely to have negative consequences for relationships in other domains (e.g., romantic; Connolly, Geller, Marton, & Kutcher, 1992), so the effects are broad.

The investigation of the relation between rejection and adolescent depression has indeed revealed a strong connection. Self-reported negative social interactions are known to lead to increases in depressive symptoms (Lee, Hankin, & Mermelstein, 2010) and experiencing rejection is highly predictive of depression during adolescence (Prinstein & Aikens, 2004). Moreover, currently depressed adolescents make more critical comments, eliciting negative emotional and behavioral reactions in their peers (Baker, Milich, & Manolis, 1996), and are less popular overall resulting in a greater likelihood of rejection (Baker et al., 1996; Connolly et al., 1992). While these findings are informative, they are largely drawn from retrospective self-report and/or behavioral data and do not specifically investigate real-time response to rejection which may in itself influence the course of depression.

To this end, several adult studies have investigated rejection-related cognitions and emotions in the context of depression. Typically in this research, the experimenters manipulated the valence of social interactions, between research participants and confederates, to force rejection (or provide negative social feedback). The measured outcomes captured emotional and/or psychological distress in response to these negative interactions. Taken together, these studies have found that, social-cognitive features of depression are associated with greater psychological distress and greater negative emotionality in response to rejection (Whittal & Dobson, 1991; Kuiper, Olinger, & Martin, 1988; Henriques & Leitenberg, 2002). Importantly these studies failed to include clinically-depressed participants. Moreover, they lack any kind of neurobiological measurement and therefore fail to provide explanation for this elevated response in terms of neural circuitry.

Taken together, investigating the neurobiological substrates of social rejection in adolescent depression may elucidate mechanisms that support the well-established rejection-

depression relationship (Platt, Kadosh, & Lau, 2013). The present study aimed to elucidate these substrates by employing the Cyberball task (Williams, Cheung, & Choi, 2000), a well-validated computer-simulated ball-tossing game with great promise for revealing the neurobiological mechanisms of rejection (Scheithauer, Alsaker, Wölfer, & Ruggieri, 2013). For the present study, the game consisted of two conditions: inclusion and total exclusion (rejection).

Aim 1: Neural response to peer rejection in depressed adolescents versus healthy controls

The first aim of the present study was to compare neural response to peer rejection in depressed adolescents as compared to healthy controls. Thus far, Cyberball has been used in several studies to examine neural response to rejection in healthy adolescents (Masten et al., 2009, 2011, 2012; Moor et al., 2010, 2012; Will, Crone, van den bos, & Güroğlu, 2013; Hillebrandt, Sebastian, & Blakemore, 2011). For instance, Masten et al. (2009) found increased insula activity and decreased ventrolateral prefrontal activity (vlPFC) in response to rejection. Activation of the subgenual anterior cingulate cortex (subACC) was related to greater levels of emotional distress, and ventral striatum (VS) activity appeared to modulate this distress (Masten et al., 2009). In a subsequent study, Moor and colleagues (2012) mapped a "response to rejection" brain circuit which included the medial prefrontal cortex (mPFC), subACC, ventral ACC, dorsal ACC, lateral PFC, and the insula. Increased insula activity was predominantly associated with rejection/exclusion. Of note, Masten et al. (2011) found rejection-related subACC activity in healthy adolescents to be predictive of parent-reported depressive symptoms one-year later, after controlling for baseline symptoms. This finding suggests subACC response may serve as a vulnerability factor for depressive onset.

The application of Cyberball to the study of adolescent depression builds on prior research that has investigated self-reported response to social rejection in depression (i.e., Kuiper

et al., 1988) by defining depressed adolescents' response to rejection in neurobiological terms. A recent investigation by Silk et al. (2013) used a virtual chat room task to examine rejection in adolescent depression and found heightened bilateral subACC and amygdala and left anterior insula activity associated with MDD. The observed increased bilateral amygdala response among depressed adolescents was thought to be at least in part attributed to anxiety comorbidity, given that anxious youth also exhibit elevated rejection-related response in this region (Guyer et al., 2009). While this study by Silk et al. (2013) is novel and significant, the experimental task has yet to be employed in multiple studies and therefore lacks the extensive body of literature supporting Cyberball. Findings from the present study may corroborate those of Silk et al. (2013), providing further evidence of atypical blood-oxygen-level dependent (BOLD) response to rejection in depression. In the present study, we expected depressed adolescents to exhibit differential neural response to rejection. Specifically, depression would be associated with greater left insula and bilateral subACC and amygdala activity in response to rejection, and reduced VS activity during social inclusion (see Moor et al., 2010).

Aim 2: Sex as a moderator of the relation between depression and BOLD response to rejection

The second aim of the present study was to examine whether sex moderated the relation between MDD and BOLD response to rejection. The potential moderating role of sex has yet to be investigated; however, several sex-related differences, in terms of social cognition, interpersonal style, social reward, and neurobiology suggest that depressed adolescent girls may exhibit particularly high BOLD response to social rejection. To begin, depression is more prevalent among females and this sex difference starts to emerge during adolescence (Rudolph, 2009). In terms of social cognition, a large body of empirical literature supports the notion that

adolescent girls are more sensitive to social cues than boys (McClure, 2000). Females have also been shown to be more sensitive to interpersonal rejection and rejection-related cues than males (Romero-Canyas & Downey, 2005), underscored by greater physiological reactivity to rejection (Stroud, Salovey, & Epel, 2002). This is likely due in part to their interpersonal style. Adolescent girls are more sociotropic, put greater emphasis on relationship-oriented goals, and are more concerned about social evaluation than adolescent boys (Rose & Rudolph, 2006). To this end, recent conceptual models of reward in depression suggest that social reward (e.g., social acceptance) may be particularly salient for girls, while boys may be more motivated by social-status-related reward (Morgan, Olino, McMakin, Ryan, & Forbes, 2013).

Finally, there are known sex differences in brain lateralization and activation patterns in the processing of social information. For instance, women demonstrated enhanced left amygdala activity when recalling negatively-valenced emotional film clips, whereas for men the right amygdala showed greater activation (Cahill et al., 2001). Adolescent girls have been found to exhibit age-related increases in neural response that are not evidenced among boys in brain regions including the insula (Guyer et al., 2009). Taken together, all of these factors converge to provide support for our expectation that depressed girls will exhibit significantly higher BOLD response than other participants in the present study. We therefore expected to find a significant female x depression interaction such that depressed girls would exhibit the greatest rejection-related BOLD response, particularly in the bilateral subACC and amygdala and left insula regions.

Implications

As it stands, the neurobiological bases of depression are not well understood, the neurobiological bases of social processes in depression even less so. Without a comprehensive

understanding of the neural underpinnings of an interpersonal disorder like depression, the field is unlikely to develop comprehensive, and more effective, approaches to treatment. The present study examined brain response to social rejection, a key mechanism in the vulnerability to and maintenance of adolescent depression. Notably, this investigation was the first to employ Cyberball and fMRI to the study of depression. While this study focused on depression exclusively, it is in support of National Institute of Mental Health (NIMH)'s initiative to create a biologically-based classification of psychopathology by establishing the methods by which the neural response to social rejection can be studied across psychological disorders.

Methods

Participants

A total of $N = 74$ adolescents were recruited for the present study. However, 3 did not participate after consenting (i.e., appointment no-show), 6 were missing scan data due to e-Prime crashing, 6 were missing self-report or interview data, 12 failed to meet inclusion/exclusion criteria for group assignment, and 12 were excluded for excessive movement during scans and/or poor scan quality. Thus, a final sample of $N = 35$ adolescents comprised two groups: depressed adolescents (Depressed, $n = 17$) and healthy control comparisons (HC, $n = 18$). Primary Diagnostic and Statistical Manual of Mental Disorders (4^{th} ed., text rev.; American Psychiatric Association, 2000) diagnoses among Depressed group participants included Major Depressive Disorder ($n = 12$), Dysthymia ($n = 1$), and Mood Disorder Not Otherwise Specified ($n = 4$). Psychiatric comorbidity predominantly included anxiety disorders: Generalized Anxiety Disorder ($n = 2$), Post-traumatic Stress Disorder ($n = 2$), Anxiety Disorder Not Otherwise Specified ($n = 5$), Social Phobia ($n = 1$), and Attention-deficit/hyperactivity Disorder ($n = 2$). Depressed adolescents were recruited shortly after admission for psychiatric treatment to the

Adolescent Treatment Program (ATP) at an inpatient psychiatric hospital serving the greater Houston, TX metropolitan area. Healthy comparisons were separately recruited from the community through a variety of methods including craigslist advertisements, local high school initiatives, and follow-ups with past lab research study participants that consented for contact.

Inclusion and exclusion criteria were as follows. Participants were required to be between 12 and 18 years of age, fluent in English, eligible for fMRI scanning, and possess adequate reading skills as determined by the Wide Range Achievement Test 4 (WRAT4; Wilkinson & Robertson, 2006). Prior to being invited to participate, HC adolescents' parents were required to complete an initial phone screen for child psychopathology (Brief Problem Monitor-Parent version, BPM-P; Achenbach & Rescorla, 2001) and their child had to score below the suggested cut-offs ($\leq$ 16 for boys and $\leq$ 13 for girls). For inclusion in present analyses, HCs were required not to report any clinically-significant elevations (*T*-Score $\geq$ 65) on the Youth Self-Report or parent-reported Child Behavior Checklist (Achenbach & Rescorla, 2001). There were two exceptions for cases in which the adolescent did not report significant YSR internalizing symptoms but their parent reported significant CBCL internalizing symptoms. Prior research indicating that adolescent internalizing symptoms are more accurately reported by the youth as opposed to parents supported the inclusion of these participants (Sourander, Helstelä, & Helenius, 1999). Depressed adolescents were required to have clinical diagnoses at admission for depressive disorders as determined jointly by staff psychologists and psychiatrists using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision (DSM-IV-TR; American Psychiatric Association, 2000), including: Major Depressive Disorder, Dysthymia, or Depressive Disorder Not Otherwise Specified (NOS). If adolescents failed to meet full inclusion criteria, had schizophrenia, any psychotic disorder, mental retardation, or

possessed less than a 5th grade reading level they were excluded. Depressed participants were not excluded due to psychiatric medications in an effort to increase generalizability of findings.

Measures

Depressive disorder diagnoses. Clinicians utilized multiple methods to determine depressive disorder diagnostic status among adolescent inpatients, including semi-structured and unstructured clinical interviews, self-report measures, and results from the NIMH Diagnostic Interview Schedule for Children Version IV (DISC-IV; Shaffer, Fisher, Lucas, Dulcan, & Schwab Stone, 2000). The DISC-IV interview is designed for use with children and adolescents between 9 and 17 years of age, and consists of several "yes/no" questions which map onto DSM-IV diagnostic criteria. Whether are not criteria are met is determined algorithmically, and therefore inter-rater reliability is not required. All DISC-IV interviews were completed by doctoral level clinical psychology graduate students or senior research assistants who completed multiple training sessions on proper administration. The DISC-IV has demonstrated high test-retest reliability for a positive diagnosis of current major depression in clinical samples (κ.92) (Shaffer et al., 2000).

Youth self-reported psychopathology. The Youth Self-Report (YSR; Achenbach & Rescorla, 2001) is a questionnaire assessing psychopathology for use with adolescents between the ages of 11 and 18 years. The YSR consists of 112 problem items capturing emotional and behavioral problems over the past 6 months, each scored on a 3-point Likert scale: 0 = *not true*, 1 = *somewhat or sometimes true*, or 2 = *very or often true*. Sample items include "There is very little that I enjoy," "I am nervous or tense," and "I am not liked by other kids." Items are summed for a variety of clinical scales, including 6 DSM-IV-oriented scales (i.e., Affective problems, Anxiety problems, Conduct problems), with *T*-score cut-offs of ≤ 65 effectively

discriminating between clinical and non-clinical respondents (Achenbach & Rescorla, 2001). Anxiety problems *T*-scores served as a covariate in primary analyses given that anxiety levels have been shown to influence brain response in depression (Warren et al., 2013; Silk et al., 2013; Guyer et al., 2009).

Parent-reported youth psychopathology. The Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001) is a questionnaire for parents' assessment of their child's emotional and behavioral problems over the past 6 months. The CBCL is for use with parents of children and adolescents between the ages of 4 to 18 years, and mirrors the items and scales of the YSR. Similarly, *T*-score cut-offs of ≤ 65 are used to discriminate between clinical and non-clinical levels of psychopathology (Achenbach & Rescorla, 2001). The CBCL was used exclusively for HC participants as an additional measure for ensuring they are free of any clinically-significant symptoms of psychopathology.

Pubertal status. The Pubertal Development Scale (PDS; Peterson, Crockett, Richards, & Boxer, 1998) is a self-report measure of pubertal status for adolescent boys and girls ages 11 and up, which can be used continuously or categorically (prepubertal to postpubertal, 5 levels). The PDS measures endocrine and somatic pubertal changes, as well as the development of secondary sex characteristics. Specifically, these changes include growth spurt in height, skin changes, and pubic hair growth for boys and girls; change in voice and facial hair growth for boys, specifically; and breast development and menarche onset in girls. The PDS therefore consists of 5 items for boys and girls, respectively. Items are rated on a four-point ordinal scale and ask whether several changes characteristic of puberty have yet to develop (*1*), have barely begun (*2*), are definitely underway (*3*), or have already been completed (*4*). The 5 items are summed and then averaged to maintain the original metric (*1 to 4*). Longitudinal research has shown the PDS

to demonstrate good reliability and criterion validity (Peterson et al., 1998; Dick, Rose, Pulkkinen & Kaprio, 2001). In the present study, continuous scores were used in analyses.

Demographics. A questionnaire was completed by parents to gather pertinent demographic information, not limited to but including: child's birth date and age, sex, racial and ethnic background, school grade, living situation, and family income. Potentially confounding demographic variables were examined and controlled for if necessary.

fMRI protocol

Experimental task. Cyberball (Williams, Cheung, & Choi, 2000) is computer-simulated ball-tossing game used to force real-time social rejection on participants (see Figure 1). The game consists of three players, the participant and two computer-simulated co-players, with one ball to toss amongst them. The game has various formats but in the present study was played under two conditions, "inclusion" and "total exclusion," each consisting of 30 ball tosses. This game version is most similar to that used by Masten and colleagues (2011). During the inclusion condition the participant received an even 33% of all ball tosses, and during total exclusion the participant received no tosses from the other two simulated players. Thus, in total exclusion, the other players shared the ball exclusively between themselves. Participants played as "Player 2" and were given unlimited time to throw the ball using a two-buttoned controller. The left button tossed the ball to Player 1 and the right button to Player 3, respectively. The duration of the first 5 throws varied between 3-3.5 seconds to allow for signal acquisition. The remaining 55 throws varied between 2.5, 3, and 3.5 seconds. Data collected during inclusion served as a baseline for measuring the neural activity in response to rejection in the exclusion condition. This well-validated task (Williams, 2007; Scheithauer, Alsaker, Wölfer, & Ruggieri, 2013) has been used to investigate neural response to rejection in several studies conducted with healthy adolescents

(Masten et al., 2009, 2011, 2012; Moor et al., 2010, 2012; Will, Crone, van den bos, & Güroğlu, 2013; Hillebrandt, Sebastian, & Blakemore, 2011).

Figure 1

Signal acquisition. fMRI scanning was performed on a 3.0 Tesla Siemens Allegra scanner. After acquisition of a high-resolution T-1 weighted anatomical scan, participants underwent whole-brain functional runs of 90 to 125 scans each for measurement of the BOLD effect (echo-planar imaging (EPI); gradient recalled echo; repetition time, 2000 msec; echo time, 40ms; flip angle, 90 degrees; 64 x 64 matrix; 24 axial slices acquired parallel to the anteroposterior commisural line, with voxels of 3.44 x 3.44 x 4 mm). Participants laid upon the scanning bed, secured and fitted with coils. Head movement was minimized by using head and chin cushions, or non-adhesive hypoallergenic tape similar to that used in hospital settings. Noise attenuating headphones were provided to reduce noise, and participants were given a squeeze ball which could be used to notify staff of discomfort and/or whether they wanted to discontinue. Blankets were provided for warmth if desired. Structural and functional imaging took place as part of a larger scan sequence that took approximately 55 minutes to complete, with approximately 2.5 minutes for the Cyberball task.

Preprocessing. fMRI data were preprocessed and analyzed in Statistical and Parametric Mapping, version 12 (SPM12; FIL Methods Group, London, UK, 2016). Slice timing correction was performed due to the bottom-up interleaved scan sequence, referencing the first slice. Realignment was performed with unwarping using interpolation of the 4^{th} degree B-spline followed by coregistration of the mean functional and anatomical images. Segmentation was performed generating an SPM12 deformation field which was in turn utilized to normalize

functional and anatomical images. Lastly, functional images were smoothed using a full-width at half maximum (FWHM) of the Gaussian smoothing kernel of 6 x 6 x 6 mm.

Preliminary analyses. For each functional run of data a protocol file was created representing the timing of each stimulus event (e.g., ball thrown to participant) which was then used to model the experiment as a block design with two conditions (inclusion and total exclusion). The "connecting" and instruction screens, which were the first 25s of functional runs, were not model nor was baseline at task conclusion. For first-level analyses, 6 motion parameters calculated during realignment (x, y, z, pitch, yaw, roll) were included as regressors of no interest. Participants whom exceeded 3 mm/degrees of movement in any direction were excluded from second-level analyses. Taking a General Linear Model (GLM) approach, group effects were evaluated using a random effects analysis with covariates centered to the overall mean. In the random-effects analysis, statistical maps were created for each participant before being subjected to second-level statistical analysis, allowing generalization to the sample. Group t-maps (one-tailed) were generated after specifying contrasts (e.g., exclusion > inclusion), and were visualized on anatomical images. Anatomical regions were labeled using the Neuromorphometrics probabilistic brain atlas accompanying SPM12.

Procedures

Upon arriving for the study parental consent was required for participation and, if provided, adolescents were asked for assent. Data was collected at one or two separate research appointments, depending on the group. Adolescent inpatients completed diagnostic assessments on the unit within a few days after admission to treatment. On separate appointments, scheduled within 7 days of diagnostic assessments, inpatient adolescents underwent fMRI scans. HCs, in turn, completed all assessments at their scan appointment.

Prior to the scan, fMRI safety instructions and procedures were reviewed with participants by research staff. Before playing Cyberball, participants' full understanding of game rules were confirmed. Since the game requires deception, debriefing occurred immediately afterwards to alleviate any psychological distress. Participants were asked to keep this aspect of the study confidential.

fMRI data analytic strategy

Aim 1: Neural response to peer rejection in depressed adolescents versus healthy controls. General Linear Modeling (GLM) was applied to first- and second-level data analyses using linear contrasts performed with and without YSR Anxiety Problems as a covariate (Warren et al., 2013). *A priori* regions of interest (ROIs) were identified based on findings from healthy adolescent Cyberball studies with emphasis on regions that may be relevant in the context of depression (Moor et al., 2012). Using the MARsBAR toolbox, ROIs were created with $8mm^3$ spheres centered at peak-voxel activations reported in prior studies. Bilateral hemispheric ROI locations were examined: subACC ([8 22 -4], [0 9 -6]); anterior insula ([38 18 -6], [-36 20 -10]); vlPFC ([27 54 9], [-44 28 10]); VS ([6 6 9], [-6 17 -2]); temporal poles ([21 -4 -32], [-24 2 -29]); and amygdalae ([-15 -3 15], [15 -3 15]) (i.e., Masten et al., 2009, 2011, 2012; Silk et al., 2013). A bonferonni corrected p-value of 0.005 was chosen for ROI statistical tests. Parameter estimates exceeding 1.5 times the interquartile range were windsorized to mitigate outlier effects. Whole-brain analyses for game contrasts were conducted with an uncorrected threshold of $p < 0.001$ (Lieberman & Cunningham, 2009); however, for *a priori* anatomical regions a less conservative threshold of $p < 0.005$ was used (Masten et al., 2012). Significant voxel activations were examined with SPM12's Neuromorphometrics probability atlas. If a voxel included more than one anatomical region, the region with the greatest probability as identified by the

Neuromorphometric atlas was chosen. All brain coordinates are reported in Montreal Neurological Institute (MNI) format.

Aim 2: Sex as a moderator of the relation between depression and BOLD response to rejection. To examine the potential moderating role of sex for anatomically-defined *a priori* brain regions, between-group linear contrasts were to be performed considering the sex x group interaction term with and without covarying for anxiety.

Results

Sample characteristics

Participants were on average age 14.80 years of age (*SD* = 1.74). The total sample was 65.7% female with a racial breakdown as follows: 63.6% Caucasian, 18.2% African American, 3% Asian and 15.2% Multiracial, with 18.2% identified as being of Hispanic ethnicity. The Depressed group was significantly older than HCs, but did not significantly differ on level of pubertal development which may have been due to missing data from approximately half of Depressed group members. There were significant differences between groups in racial composition but not Hispanic ethnicity. As expected, the Depressed group reported significantly greater symptoms of depression and anxiety. While groups did not differ on sex, their respective sample sizes paired with the within-group sex distributions, however, prevented the examination of sex as a moderator due to limited power. Given that groups significantly differed on age it served as a covariate for age in primary analyses. For descriptive statistics and group comparison results, see Table 1.

Table 1

Whole-brain analyses

See Table 2 for significant results from between-group whole-brain voxel-wise analyses controlling for age. The Exclusion > Inclusion contrast was of foremost interest. Partially supporting hypotheses, the Depressed group was found to exhibit significantly greater activation than HCs in several anatomical regions including voxels in the right anterior insula and left nucleus accumbens (ventral striatum) (see Figure 2). Left anterior insular activation failed to reach statistical significance ([-46 2 -2], $t = 2.64$, $p = 0.0064$, $k = 81$). The Inclusion > Exclusion contrast showed HCs to in turn exhibit significantly greater voxel activation in the right precuneus and right middle cingulate gyrus. Examining Exclusion and Inclusion blocks independently revealed the Depressed group to exhibit significant activation in the left and right anterior insula in both game conditions; however, as shown in the Exclusion > Inclusion contrast results, the right anterior insula specifically was differentially activated in Exclusion. HCs showed no significantly greater whole-brain activation in the Inclusion block. Analyses were repeated controlling for YSR anxiety symptoms, with few regional activations remaining statistically significant (see Table 2). Of note, the left nucleus accumbens remained more active in the Depressed group in Exclusion > Inclusion whereas right anterior insular activation was no longer significant. Taken together, across whole-brain analyses there were non-significant between-group activations in the bilateral subACC, vlPFC, and amygdalae.

Table 2, Figure 2

ROI analyses

ROI analyses using extracted mean cluster-level parameter estimates for participants from previously identified peak-voxel coordinates were then performed. Consistent with our approach to whole-brain data, age served as a covariate and analyses were subsequently repeated

covarying for anxiety symptoms. With a Bonferroni-corrected significance threshold of $p < 0.005$, no ROI-contrast analyses reached significance. Exploratory analyses with a greater threshold of $p < 0.01$ resulted in one significant region across contrasts: left vlPFC, $t = 2.866$, $p = 0.007$. When controlling for anxiety, results remained the same.

Testing for puberty x group interactions

Despite PDS data missing from 9 participants (8 depressed), exploratory analyses were performed to examine whether any significant puberty x group interactions were evident at the whole-brain level or ROIs for the primary contrast of interest, Exclusion > Inclusion. For whole-brain analyses, in model specification, dimensional PDS scores were entered as a covariate interacting with the group factor. The Exclusion > Inclusion contrast revealed non-significant voxel-wise results for *a priori* brain regions. For ROI analyses, separate univariate GLM models were tested for group and PDS main effects and group x PDS interactions with each respective ROI serving as a dependent variable. All findings were non-significant with interaction term *p*-values ≥ 0.209.

Discussion

The present study was the first to employ Cyberball and fMRI to examine rejection-related brain response in clinical depression within any age group. Between-group whole-brain voxel-wise comparisons revealed depressed adolescents to exhibit significantly greater rejection response in several anatomical regions including the right anterior insula, left ventral striatum (nucleus accumbens), and left parietal operculum. During inclusion, depressed adolescents exhibited significantly greater activation in the right middle cingulate cortex and the precuneus, an integratory region implicated in self-referential processing (Cavanna & Trimble, 2006). Also, depressed adolescents exhibited similar ventral striatal inclusion response. Contrary to

expectations, ROI analyses for increased between-group rejection response in the bilateral subACC, anterior insula, amygdalae, vlPFC, ventral striatum, and temporal poles went unfounded. Nevertheless, whole-brain findings may help further current understanding of a salient social mechanism in the onset and maintenance of adolescent depression. Taken together, study hypotheses received mixed support and limited sample sizes and incomplete data leave important questions for future studies.

The fact that depressed adolescents did not exhibit heightened activity in the subACC and amygdala was surprising yet makes sense in greater context. The hypothesized role of the subACC was supported by Masten and colleagues (2011) finding that subACC response to social rejection predicts later depressive symptoms among healthy adolescents. If subACC processing indeed confers risk for depression, it was expected that it would be significantly more active in depressed adolescents, potentially serving as a trait-marker. However, Masten et al. (2011) also found that subACC response did not associate with concurrent depressive symptoms, which suggests that a protracted relation may exist. Further, depressed adolescents in the present study showed greater rejection activity in the middle as opposed to anterior cingulate. Though a question for future longitudinal investigations, perhaps a shift between anterior and middle cingulate recruitment in response to rejection occurs with depressive onset.

Heightened bilateral amygdala response was expected as it has been observed in other social rejection-adolescent depression research (Silk et al., 2013). However, that study employed a virtual chatroom task with potentially greater ecological validity than Cyberball. In that instance, the adolescent participant is engaged in a pseudo-social media-like environment interacting with peers with names and faces provided. In these exchanges, amygdala responsivity may in part be a function of encoding emotional salient information about these peers (see

Phelps & LeDoux, 2005). Moreover, fear of social ostracism extending beyond a one-to-one peer interaction may be triggered in this context as the task is similar to mediums like Facebook, which connect users in a network that extends well beyond face-to-face daily interaction (Ellison, Steinfield, & Lampe, 2007). Rejection in Cyberball, a cartoon game played with strangers, may be experienced as a more isolated, less interpersonal event. One further consideration is that increased amygdala response to positive social feedback (social evaluation) has also been shown in depressed adolescents (Davey, Allen, Harrison, & Yücel, 2011). Perhaps amygdalar response is driven by the social evaluative nature of these tasks rather than valence of provided feedback. Whereas the subACC and amygdala were not identified in the present study, findings implicate the right anterior insula and left nucleus accumbens.

The anterior insula has been implicated in numerous facets of the human condition including body awareness, interoception, self-recognition, the perception of time, and emotional awareness (Craig, 2009). The sheer breadth of functioning and behaviors associated with the insula make it difficult to isolate one or a few dominant processes that may be associated with depression. Though what may be a highly relevant finding comes from the neuroeconomic literature. Harlé, Chang, van't Wout, and Sanfey (2012) used a social economic exchange game with sad mood induction in healthy adult participants. The authors found sad participants to exhibit significantly greater anterior insular responsivity to perceived unfairness during social exchanges and, moreover, this activation mediated the relation between sadness and social decision-making. This finding demonstrates an integral role of the bilateral anterior insula in transient sadness, with subsequent implications for real-time, real-word social behavior. Anomalous anterior insular neural activity and similar social consequences would be expected to extend to chronic depression (Mellick, Sharp, & Ernst, 2015). Given the multitude of insular

functions, other components of the depressive experience beyond negative emotion and disrupted social interaction (i.e., abnormal interoception; Avery et al., 2014) may also be influenced by aberrant insular processing. To this end, the depth and breadth in which the anterior insula may be implicated in depression seems paramount and dysfunction may not be unique to depression. In fact, a recent meta-analysis of structural neuroimaging studies identified the anterior insula as a key component of a broader neural network that appears to be implicated as a shared neural substrate of several psychopathologies (Goodkind et al., 2015). When anxiety symptoms were controlled for in group comparisons, the right anterior insula activation we observed in depressed > healthy adolescents failed to retain significance which may support this proposition.

The fact that we found *right* anterior insular activation to differentiate depressed versus healthy adolescents stands in contrast to the aforementioned virtual chatroom study by Silk and colleagues (2013) which showed *left* anterior insular response to associate with adolescent depression. However, our findings align in that healthy adolescents did not exhibit significant anterior insula activation in the rejection condition. Present findings are also consistent with Silk et al. (2013) in that depressed participants did not show reduced nucleus accumbens activation to inclusion/acceptance and instead showed heightened response in that region to rejection. The former finding, of similar social reward processing (inclusion) between groups, was contrary to hypotheses and deviates from the extant reward literature of depression. This highlights the continued need for investigation of reward function in adolescent psychopathology (Forbes & Goodman, 2014). As suggested by Silk et al. (2013), elevated nucleus accumbens response to rejection may associate with stronger neural encoding of aversive or punishing experience.

There is a range of plausible explanations for why ROI analyses resulted in null findings. Though there are numerous strengths to ROI analytical approaches, they are not without

limitations. One of which that may be relevant in this instance is the assumption of functional homogeneity among voxels within anatomical regions (Poldrack, 2007). While an alternative approach could have been extracting ROIs from functional activations among present study participants, we elected to utilize peak-voxel MNI coordinates identified in other Cyberball fMRI studies (Masten et al., 2009, 2011; Moor et al., 2012). Statistically-insignificant results may have resulted from the fact that these coordinates were drawn from healthy samples which, in turn, may not be uniformly applicable to psychiatric populations. For instance, the subACC voxels have been repeatedly activated in prior work yet depressed adolescents in the present study showed significant middle cingulate voxel activation when within-group second-level analyses were examined. Additionally, it is plausible that even if the same anatomical regions are implicated across populations, perhaps peak-voxel activation differs which may suggest functional heterogeneity. This was evidenced by the non-significant ventral striatal ROI result but significant voxel activation in the nucleus accumbens, albeit at a different coordinate.

An alternate explanation for null ROI results, though highly speculative, stems from the possibility that depressed adolescents may play the game differently such that their subjective experience of Cyberball rejection may differ from healthy adolescents. Thereby providing a rationale for why expected neural regions were not significantly more active. This potentially altered subjective reaction in which different neural regions are recruited may relate to the fact that adolescents with depressive onset often have prior in-vivo interpersonal rejection experiences (i.e., adolescent romantic loss; Monroe, Rohde, Seeley, & Lewinsohn, 1999). Another commonly used rejection paradigm has participants relive a recent, unwanted breakup and elicits different neural responses than Cyberball. For example, meta-analyses show the bilateral anterior insula is reliably activated by Cyberball; however, this “reliving the breakup”

paradigm reliably activates the right anterior insula alone (Cacioppo et al., 2013). Depressed adolescents in the present study similarly showed right but not left anterior insular activity in response to Cyberball rejection. That being said, perhaps when the depressed adolescents began experiencing Cyberball rejection rumination about prior rejection experiences (i.e., romantic loss) may have occurred, hence the right insular dominance. The long duration of the exclusion block (75-90s) may have permitted such mental drift.

A final consideration to this point is that Cyberball did not necessarily fail to elicit neural response in the subACC, vlPFC, amygdalae, and temporal poles in depressed adolescents. Rather, the activation was simply no greater among them than observed in healthy controls. Therefore, findings suggest that some components of the rejection-related brain circuit are disrupted in adolescent depression rather than the circuit in its entirety. Hypotheses, in this respect, were guided by an all-or-nothing approach which in turn required substantial Bonferroni correction.

While this study has several strengths it is not without limitations. There have been doubts expressed about whether Cyberball measures response to rejection or social pain, specifically; moreover, rejection from complete strangers may be insufficient to activate true social pain (Cacioppo et al., 2013). However, the task has been used extensively in the field, converging on a cohesive pattern of findings among typical healthy adolescents. Various game formats have been used across these studies with mean ostracism effects comparable regardless of number of ball tosses or duration of blocks (Hartgerink, van Beest, Wicherts, & Williams, 2015). Thus, with adolescents prone to movement during scanning, we may have improved fMRI data quality and participant investment in the task had we utilized a shorter game. Using a self-report manipulation check in conjunction with the Cyberball task would have been preferable but

was not included because a substantial portion of scan data had been collected prior to the initiation of the present study. Potential task-related measures could have included pre- and post-game, such as the Need-Threat Scale (NTS; Williams et al., 2000) and mood questionnaires. Assessment of HCs could have been strengthened had structured clinical interviews been administered; however, staffing and time restraints prohibited them from the battery. Nonetheless, the YSR and CBCL possess strong psychometrics and have been shown to discriminate between clinical and nonclinical samples (Achenbach & Rescorla, 2001). Moreover, dual reporters (adolescents and parents) were utilized. Handedness data were also missing for depressed adolescents; however, 75% of healthy recruits were known to be right-hand dominant. Furthermore, a majority of depressed participants had psychiatric medication history prior to assessment and power limitations precluded covarying for potential effects. Recruitment efforts fell short of attaining adequately large sample sizes to provide sufficient power to test sex as a moderator of the relation between depression and BOLD response to rejection. Thus, this important research question stands for future studies; however, the challenges of examining sex as a moderator in fMRI research remain (Button et al., 2013). Furthermore, although the intention was to match participants on pubertal status and sex it was not feasible due to missing PDS data along with the female preponderance of depression paired with limited sample sizes. In attempt to account for this shortcoming, follow-up exploratory pubertal x group interaction tests were performed though they yielded non-significant results.

In hope, statistically significant and socially meaningful socioeconomic and racial/ethnic group differences that went uncontrolled for in the present study provides impetus for future investigations to consider these variables as rejection-response moderators in depression. Recent research highlights the value of such work. For instance, adolescents living in low-

socioeconomic neighborhoods have shown greater neural response to rejection in the dorsal anterior cingulate cortex in young adulthood compared to more economically-advantaged comparisons, irrespective of self-reported rejection distress (Gonzalez, Beckes, Chango, Allen, & Coan, 2015). Perceived racial prejudice in Cyberball rejection has also been examined and revealed more profound emotional distress when White and African American players were told their co-players were of the opposite race (Goodwin, Williams, & Carter-Sowell, 2010). Considering how these variables may interact with depression in modulating neural rejection response presents an exciting opportunity for future neuroimaging research. Furthermore, it would provide an indirect means to reduce the degree of underrepresentation of diverse racial and ethnic groups in clinical neuroimaging research (Isamah et al., 2010).

Conclusions and future directions

This was the first Cyberball-fMRI investigation of adolescent depression and extends knowledge of rejection in depression beyond self-report and behavioral data by helping define rejection response in terms of neural circuitry. The present study is among the few that recently utilized this methodology to study psychiatric populations marked by interpersonal functioning deficits (Domsalla et al., 2014; Nishiyama et al., 2015). By continuing such efforts we may help delineate psychological disorders and/or reveal shared neural substrates of key interpersonal processes. As a larger proportion of studies have been with adults, there remains a greater relative need for extending this line of work to adolescents which is arguably magnified by the salience of social processes during adolescence.

The discussion above underscores the need for a more robust study of rejection response in terms of populations, age groups, and methodology. While the Cyberball task has substantial merit in the field (Platt et al., 2013), alternative tasks with greater ecological validity, particularly

in the context of adolescent depression, may yield more true-to-life neural processing. The aforementioned virtual chatroom and romantic breakup tasks potentially tap into more salient social processes that are related to adolescent depression on a more meaningful level. Furthermore, important sex differences in the salience of reward types may provide an opportunity for customization in study designs. For instance, adolescent girls are suggested to respond more to affiliative reward whereas adolescent boys may be more responsive to status-related reward (Morgan et al., 2013). Thus, utilizing individualized reward types for research participants may provide more relevant and compelling results.

Finally, present findings demonstrate significant roles of the right anterior insula and left nucleus accumbens in depressed adolescents' rejection response. However, the occipital operculum was also significantly more activated in adolescent depression. This region supports pain modulation with connections to the insula so it may also be relevant for conceptualization of depression (Kong et al., 2010). Future rejection studies may replicate and build on this finding by examining functional connectivity among these regions and employing an array of within- and between-subject analyses using multiple levels of neuroimaging.

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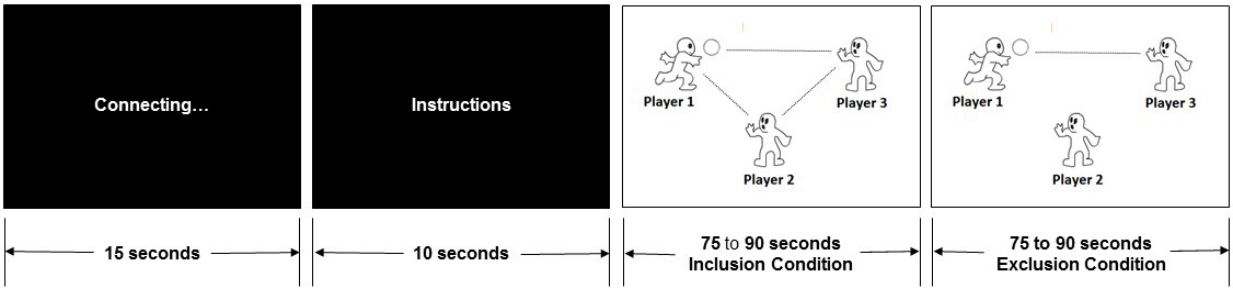
Figure 1. Cyberball task



Participants were presented with a "Connecting…" screen (15s) followed by game instructions (10s). Inclusion always preceded exclusion with each condition lasting approximately 75 to 90s. Condition duration varied within- and between-subjects as a function of player response times and random duration of ball tosses.

Table 1. Sample characteristics and group comparison results

	Depressed (n = 17)	**HC** (n = 18)	t/χ^2	p
Age	15.53 (1.46)	14.11 (1.74)	2.598	0.014
Sex (%female)	76.5	55.6	1.697	0.193
PDS	3.53 (0.45)	3.15 (0.80)	1.568	0.130
YSR Affective	72.53 (13.20)	52.00 (2.89)	5.907	< 0.001
YSR Anxiety	65.67 (9.51)	51.83 (3.71)	5.304	< 0.001
Race			17.612	< 0.001
African American	-	6 (33.3%)		
Caucasian	16 (94.1%)	5 (27.8%)		
Asian	1 (5.9.%)	-		
Multiracial	-	5 (27.8%)		
Hispanic (%yes)	5.9	27.8	3.812	0.051
Household income	170.50K (19.8K)	30.67K (20.6K)	12.08	< 0.001

Note: Data are mean (standard deviation) aside from sex, race, and Hispanic ethnicity. Groups compared using independent samples t-tests, Chi-square tests of independence, and Fisher's Exact test. YSR = Youth Self Report; PDS = Pubertal Development Scale. Household income reported in annual U.S. dollars. Missing data on race/ethnicity (HC, n = 2), the YSR (Depressed, n = 2), and PDS (Depressed, n = 8; HC, n = 1).

Table 2. Depressed > HC between-group voxel-wise activations controlling for age

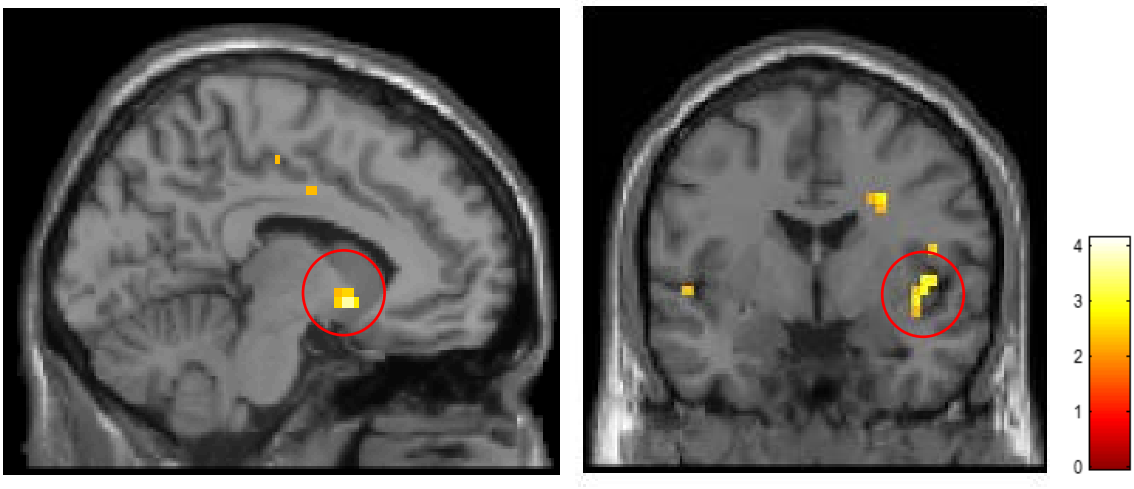
Anatomical Region		*t*	*k*	*x*	*y*	*z*	*p*
Exclusion > inclusion							
Middle temporal gyrus	R	4.14	101	61	-50	6	< 0.0005
Middle occipital gyrus	R	3.72	101	33	-74	10	< 0.0005
Parietal operculum	L	3.81	81	-53	-26	22	< 0.0005
Superior temporal gyrus	R	3.67	61	57	-26	6	< 0.0005*
Planum temporale	R	3.51	61	64	-23	14	< 0.001
Accumbens	L	3.61	20	-8	8	-6	< 0.001*
Cerebellum	R	3.37	20	37	-67	-34	< 0.001*
Anterior insula	R	2.99	74	44	-2	2	< 0.005
Inclusion > exclusion							
Precuneus	R	3.72	355	9	-60	38	< 0.0005*
Middle cingulate gyrus	R	3.58	355	9	-26	26	< 0.001*
Hippocampus	L	3.62	120	-25	-33	-6	< 0.001
Cerebellum	L	3.58	95	-8	-57	-34	< 0.001*
Exclusion only							
Planum polare	L	3.62	2457	-46	2	-6	< 0.001
Central operculum	L	3.40	2457	-42	-5	10	< 0.001
Middle cingulate gyrus	L	3.42	90	-15	-12	42	< 0.001
Anterior insula	L	3.25	2457	-39	5	-14	< 0.005
Anterior insula	R	3.05	2457	44	8	-10	< 0.005
Caudate	R	3.05	2457	9	15	14	< 0.005

Temporal pole	R	2.78	2457	54	8	-26	< 0.005
Inclusion only							
Cerebellum	L	3.75	59	-11	-64	-34	< 0.001
Fusiform gyrus	R	3.70	1009	37	-57	-18	< 0.001
Precentral gyrus	R	3.58	162	37	-5	38	< 0.001
Central operculum	L	3.57	2791	-42	-5	10	< 0.001
Planum polare	L	3.45	2791	-46	2	-6	< 0.001
Anterior insula	R	2.83	2791	44	8	-14	< 0.005
Anterior insula	L	2.81	2791	-39	8	2	< 0.005

A priori regions are listed if significant at $p < 0.005$. All other active regions are listed if significant at $p < 0.001$ uncorrected. Height thresholding set to T = 1.69 ($p < .05$ uncorrected) with extent thresholding at 0. R = right atmosphere; L = left atmosphere; *k* = number of voxels in cluster; Coordinates (*x*, *y*, *z*) in MNI space.

*Remained significant after controlling for YSR anxiety symptoms. *Note*: Two depressed participants were missing YSR data so analyses performed with subsample.

Figure 2. Greater left nucleus accumbens and right anterior insula activation in depressed adolescents



Exclusion > inclusion. Significantly greater response in Depressed > HC in left accumbens ($p < 0.001$ [-8 8 6]) and right anterior insula (*a priori* defined $p < 0.005$ [44 -2 2]) controlling for age. Height threshold set to $p = 0.020$ for diagrammatic purposes.

Appendix A

Power analyses

The present study most closely resembled that of Masten et al. (2011) which examined group differences in rejection-related neural activity in adolescents with autism spectrum disorders versus healthy controls. Using traditionally accepted standards for determining statistical power in neuroimaging research (Desmond & Glover, 2002) with G-Power software (Faul et al., 2007), desiring a large effect size (d = .80) while setting alpha to 0.05 and power ($1 - \beta$) to 0.95 required the total sample size to be N = 130. However, this size sample was ambitious and also calculated with the goal of testing for sex as a moderator. Though recruitment efforts fell short, the N = 35 participants retained for analyses is rather consistent with that of the broader fMRI literature (Button et al., 2013).