Investigating the Link between Neural and Real-World Responses to Social Threat in Adolescents at High Risk for Social Anxiety

by

Stefanie Lee Sequeira

Bachelor of Science, University of Maryland, 2014

Submitted to the Graduate Faculty of the

Dietrich School of Arts and Sciences in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2021

UNIVERSITY OF PITTSBURGH

DIETRICH SCHOOL OF ARTS AND SCIENCES

This thesis was presented

by

Stefanie Lee Sequeira

It was defended on

January 14, 2019

and approved by

Jennifer S. Silk, PhD, Department of Psychology

Cecile D. Ladouceur, PhD, Department of Psychiatry

Jamie L. Hanson, PhD, Department of Psychology

Thesis Advisor: Jennifer S. Silk, PhD, Department of Psychology

Investigating the Link between Neural and Real-World Responses to Social Threat in Adolescents at High Risk for Social Anxiety

Stefanie Lee Sequeira, MS

University of Pittsburgh, 2021

Adolescence is associated with increases in sensitivity to social evaluation, which may be supported by normative developmental changes in brain function. However, heightened neural reactivity to negative social evaluation, such as peer rejection, may place adolescents at increased risk for social anxiety. The present study used novel, ecologically valid methods to test the hypothesis that heightened neural reactivity to peer rejection is associated with symptoms of social anxiety in early adolescent girls. Further, we examined whether this association might be explained by heightened emotional reactivity to social threat in daily life. Ninety-nine adolescent girls (ages 11-13 years) oversampled for shy/fearful temperament, a risk factor for future social anxiety, completed a 16-day ecological momentary assessment protocol in which they reported on their emotional responses to daily negative experiences with peers (i.e., daily experiences of social threat). Following this assessment, girls completed a social interactive task, in which they were accepted or rejected by their peers and completed control trials, in a magnetic resonance imaging scanner. Girls also reported on their social anxiety symptoms. Brain regions that activated significantly to peer rejection relative to either peer acceptance or a control were tested as predictors of social anxiety symptoms and daily experiences of social threat. Associations between neural activation to peer rejection (relative to acceptance or a control) and social anxiety symptoms were not supported. However, activation in the left caudate nucleus to peer rejection (relative to a control) was significantly associated with daily experiences of social threat, and a significant

indirect effect of daily experiences of social threat on the association between left caudate activation to peer rejection and social anxiety symptoms was found. These associations were not significantly moderated by perceived friendship quality or pubertal status. Findings may suggest that adolescent girls with higher caudate activity to rejection are more likely to attend to and recall social threatening interactions, and that greater recall of social threat is associated with social anxiety. However, the cross-sectional design limits any causal interpretations that can be drawn from the indirect effect model. Future research is needed to test these questions using a longitudinal design.

Table of Contents

List of Tables

List of Figures

Acknowledgments

This project was supported by National Institute of Mental Health grant R01 MH103241 (PI: J.S. Silk and C.D. Ladouceur) and a National Science Foundation Graduate Research Fellowship awarded to S.L. Sequeira under Grant No. 1747452.

1.0 Introduction

Understanding how peer interactions influence the development of social anxiety is of central importance in adolescence. Adolescence is a developmental period characterized by heightened sensitivity to social cues, in which peer relationships are especially important (Guyer et al., 2012). Accompanying this normative increase in social sensitivity, however, is an increased risk for developing social anxiety (Merikangas et al., 2010). Substantive literature has shown that peer rejection is associated with the development of psychopathology in adolescence (Platt et al., 2013; Kingery et al., 2010; La Greca & Harrison, 2005), though the presence of close friends may attenuate this association (Hodges et al., 1999; Peters et al., 2011). Although the link between peer rejection and psychopathology is well established, additional research is needed to better understand how this association occurs. This research is particularly critical for adolescent girls, as they are more sensitive to social evaluation (La Greca & Lopez, 1998; Rudolph & Conley, 2005) and are at increased risk for developing social anxiety compared to adolescent boys (Merikangas et al., 2010).

Recent work has applied functional magnetic resonance imaging (fMRI) methods to explore potential neural correlates of peer rejection that may influence the development of social anxiety. Studying adolescent brain function in the context of peer relationships is important, as significant changes in cognitive and affective brain regions are occurring during this developmental period that may be particularly sensitive to social input (Blakemore & Mills, 2014). This research has shed light on how one mechanism, increased activation in a social-affective neural network during peer rejection, influences the development of social anxiety in adolescence (Rudolph et al., 2016). However, research has not yet linked this heightened neural activation to

real-world affective responses to peer rejection; this link is generally assumed but has not been directly tested in adolescents. The central aim of the current study was to examine how neural activation to peer rejection in a social-affective neural network relates to real-world experiences of peer rejection, and how these neural and real-world measures relate to concurrent symptoms of social anxiety. We were also interested in how the presence of close friendships and pubertal development influences these links. Examining how close friendships moderate the relationships between neural activation to rejection, real-world social threat, and social anxiety symptoms may be important for understanding the mechanisms through which close friendships work to protect adolescents experiencing peer rejection from developing psychopathology. Finally, when studying social-affective neuroscience and psychopathology in adolescents, it is important to consider the role of pubertal development, as the hormonal and neural changes associated with puberty contribute to both social changes and growing rates of anxiety disorders in this population (Mendle, 2014).

1.1 Adolescence as a risk period for social anxiety

Adolescents spend more time interacting with peers than do young children or adults, and they commit extensive time and energy to forming peer networks and trying to win peer approval (Steinberg & Morris, 2001). Positive peer experiences can promote social and emotional development in adolescence (Festa & Ginsburg, 2011) and social support from friends can enhance self-esteem and academic achievement (Cohen & Wills, 1985). However, negative peer experiences, such as peer rejection and victimization, can have harmful influences on adolescent

well-being and contribute to psychopathology (Platt et al., 2013; Kingery et al., 2010; La Greca & Harrison, 2005).

Importantly, adolescence represents the highest risk period for the onset of social anxiety symptoms (Merikangas et al., 2010). Social anxiety in adolescence predicts underachievement in school, smaller social networks, and poorer social skills (Kashdan & Herbert, 2001; Ginsburg et al., 1998). Youth with social anxiety can also suffer from high levels of loneliness, dysphoria, and generalized anxiety (Beidel, Turner, & Morris, 1999) and often have comorbid depressive disorders and substance use disorders (Essau, Conradt, & Petermann, 1999). Further, even shy adolescents (not clinically anxious) are at risk for suicide attempts (see Zimbardo & Radl, 1981).

Existing research strongly suggests that adolescents who experience more peer rejection and victimization experience higher levels of affective distress, loneliness, and anxiety (Kingery et al., 2010; La Greca & Harrison, 2005). Research has shown that children who perceive themselves as more socially accepted report lower levels of social anxiety (Festa & Ginsburg, 2011), while highly socially anxious children and adolescents report lower levels of social acceptance and more negative peer interactions (Ginsburg, La Greca, & Silverman, 1998; Erath, Flanagan, & Bierman, 2007). Further, adolescent girls with high social anxiety report having fewer close friends and perceive existing friendships as being lower in intimacy, companionship, and support than adolescent girls with lower levels of social anxiety (Velting & Albano, 2001).

In an experimental attempt to link physiological responses to peer rejection with feelings of loneliness in adolescents, Silk et al. (2012) measured feelings of social connectedness to peers in daily life (a proxy for loneliness) using an Ecological Momentary Assessment (EMA) protocol administered over three consecutive weekends. In the laboratory, the authors also measured physiological arousal (via pupillary response) to peer acceptance and rejection during the

Chatroom Task, a task in which participants believe they are being chosen (peer acceptance) or not chosen (peer rejection) by their peers to discuss certain topics. The authors found that increased physiological arousal to the laboratory measure of peer rejection in adolescence was associated with lower feelings of social connectedness with peers in daily life (Silk et al., 2012).

1.2 Using fMRI to study sensitivity to peer rejection in adolescence

Recent research has also used fMRI to examine neural responses to peer rejection. These studies have typically employed two paradigms: a Cyberball virtual ball-tossing task (Eisenberger et al., 2003; Rudolph et al., 2016) or a version of the Chatroom Task (Guyer et al., 2008; Silk et al., 2014). Findings from these studies in adolescents show that peer rejection activates a social-affective network that includes the amygdala, medial prefrontal cortex (mPFC), ventrolateral prefrontal cortex (vlPFC), dorsal and subgenual anterior cingulate cortex (dACC, sgACC), nucleus accumbens (NAcc; part of the striatum), and anterior insula (Eisenberger et al., 2003; Guyer et al., 2008; Masten et al., 2009; Lau et al., 2012; Silk et al., 2014; Will et al., 2016). This network is critical for interpreting social cues and regulating or inhibiting affective responses to these cues (Jarcho et al., 2013).

Heightened activation to peer rejection in this social-affective network is consistent with developmental findings that neural responses to social evaluation increase normatively during adolescence. Several studies have found increases in neural response to peer evaluation in regions of the affective processing network, including the amygdala, insula, striatum, mPFC, and ACC, during the transition from childhood to adolescence (Guyer et al., 2009; Gunther Moor et al., 2010; Bolling et al., 2011). While some heightened activation in this network may be developmentally

normative, research has begun to link heightened activation in this network to social anxiety in adolescence. In a study using the Chatroom Task with fMRI, Guyer et al. (2008) found greater amygdala activation in socially anxious adolescents compared to non-anxious adolescents when anticipating potential peer rejection. In a nonclinical sample, Rudolph et al. (2016) found that greater activation in the dACC, sgACC, and anterior insula during peer exclusion predicted higher levels of social anxiety. Therefore, although heightened activation in the social-affective neural network may be normative in adolescence, the response of some regions in this network to peer rejection relates to both clinical and subclinical symptoms of social anxiety.

A few recent studies have also examined how adolescents' experiences with peer victimization relate to neural activation to social exclusion and current internalizing symptoms. For example, Will et al. (2016) found that a history of chronic childhood peer rejection (measured using peer-nomination procedures) was associated with increased activation in the dACC during social exclusion in adolescence. In a second study examining the relationship between peer rejection and internalizing symptoms, Rudolph et al. (2016) found that, in adolescent girls, the relationship between neural response to peer exclusion and internalizing symptoms was moderated by victimization status (measured using girls' self-reports of victimization from 2nd through 8th grades), such that this relationship was stronger in chronically victimized than non-victimized girls.

Similarly, additional research suggests that individuals higher in rejection sensitivity experience increased neural activation to social exclusion. In one study in adults, Burklund et al. (2007) found that adults with greater self-reported rejection sensitivity showed greater dorsal ACC activation in response to viewing disapproving faces, but not in response to viewing angry or disgusted faces. The authors suggest that individuals with higher rejection sensitivity are also more

sensitive biologically to facial expressions signaling rejection, but not to threat in general (Burklund et al., 2007). In a comparable study in adolescents, Masten et al. (2009) found that insula and sgACC activation to peer rejection positively correlated with self-reported distress immediately following the fMRI task. Additionally, replicating the findings of Burklund et al. (2007), greater sgACC activation to rejection positively correlated with self-reported rejection sensitivity (Masten et al., 2009). These results suggest that general self-reported sensitivity to rejection does map on to neural sensitivity to rejection in the laboratory.

Limited research has also attempted to link neural responses to peer rejection to adolescent behavior among peers. In a study using Cyberball to examine neural correlates of social rejection in mid-adolescents, Sebastian et al. (2011) found increased activation in the mPFC, sgACC, OFC, and vlPFC during social exclusion. Further, the authors reported that greater activation in the mPFC during exclusion compared to inclusion was associated with greater self-reported susceptibility to peer influence (Sebastian et al., 2011). The authors interpret this latter finding as suggesting that the neural response to social rejection relates to adolescent behavior in the real world. However, susceptibility to peer influence is a subjective measure, and it is impossible to know just how much this measure maps onto real, dynamic peer relationships.

Importantly, while these previous studies have linked neural activation in this social-affective network to measures of victimization status, rejection sensitivity, and susceptibility to peer influence, no fMRI study has yet linked neural activation in this network to concurrent self-reported experiences of peer rejection in children or adolescents; this work has only been done in adults (Eisenberger et al., 2007). This is a major gap in the fMRI literature, as it is impossible to know from existing research whether adolescents who show heightened social-affective neural responses to peer rejection in the laboratory also show heightened affective responses to real-world

experiences of social threat. Further, this link is likely bidirectional, such that adolescents who show heightened affective responses to social threat in daily life also experience greater neural sensitivity to social threat. Using EMA, which provides information closer to real-time, may help better elucidate how neural activation to rejection relates to specific affective responses to peer rejection in daily life.

1.3 Linking fMRI and EMA to study sensitivity to peer rejection in adolescence

Recent research has made promising initial attempts to link fMRI and EMA (e.g., Masten et al., 2012; Forbes et al., 2009; Wilson et al., 2014; Heller et al., 2015; Price et al., 2016; Fischer et al., 2017), although this link is still largely unexplored. EMA studies are unique in that they allow one to study behavioral, affective, and situational variables with rich insight in naturalistic conditions (Wilson et al., 2014). However, even though EMA reduces retrospective bias, it still relies on self-report. Further, individuals may not be able to accurately express their affective states via EMA (Wilson et al., 2014). An advantage of fMRI is that it taps a more objective, biological phenomenon and assesses affective processing nonverbally, which makes it immune to problems that typically plague self-report measures (Wilson et al., 2014). fMRI aims to study neural mechanisms that guide behavior, however the links between neural reactivity and real-world behavior have rarely been examined directly (Wilson et al., 2014; Hasson & Honey, 2012), thus the ecological validity and clinical relevance of much fMRI research is often unclear. Indeed, EMA and fMRI are complementary approaches with unique strengths that tap different components of affective processes. Integrating EMA and fMRI allows one to bridge brain in the laboratory and

behavior in more naturalistic settings, to potentially increase the generalizability and clinical relevance of both fMRI and EMA findings (Wilson et al., 2014).

In one of the first and only studies attempting to link fMRI response to social threat to real-world experiences of social threat using EMA, Eisenberger and colleagues (2007) found that adults who showed greater activation in regions associated with processing social threat (i.e., dorsal ACC, amygdala, periaqueductal grey) during an fMRI social rejection task reported feeling greater momentary social distress in daily life. Further, individuals who showed greater activation in regions involved in memory encoding (i.e., hippocampus, mPFC) showed a stronger positive correlation between measures of momentary social distress and measures of end-of-day social disconnection (Eisenberger et al., 2007). In a comparable longitudinal study in adolescents, Masten et al. (2012) used daily diary methods and fMRI to show that greater involvement with friends in daily life was associated with less neural sensitivity to social rejection in young adulthood. This study, however, focused on social reward in daily life; no neuroimaging study has yet examined the link between real-world experiences of social threat and neural response to peer rejection in children or adolescents.

1.4 Friendship quality and pubertal status as potential moderators

An additional variable that may be important to consider when examining the link between peer rejection and social anxiety is friendship quality. Behavioral research, and limited neural research, suggests that peer friendships in adolescence may act as a buffer on the negative effects of peer rejection and victimization. Adolescents with a close friendship providing social support may be able to cognitively appraise experiences of rejection from other peers less negatively, and

thus internalize this rejection less, than adolescents without a close friend to provide that social support. Indeed, studies have shown that the positive association between peer victimization and internalizing symptoms is attenuated in children who report a close, protective relationship with a best friend (e.g., Hodges et al., 1999). In one such study examining physiological effects of peer rejection, Peters et al. (2011) found that children who experienced more social exclusion had elevated cortisol levels at school, but this effect was weakened by higher levels of reported friendship quality. Another study in adolescents found that positive qualities in best friendships protected adolescents against social anxiety, while negative interactions in best friendships predicted high social anxiety (La Greca & Harrison, 2005). Finally, Masten and colleagues (2012), using a daily diary approach and fMRI, found that greater involvement in friends during adolescence was associated with less neural sensitivity to social threat in young adulthood. Specifically, the authors showed that greater friend involvement in adolescence was associated with less activity in the dorsal ACC and anterior insula two years later while experiencing peer rejection during an online ball-tossing game (Masten et al., 2012). Existing neuroimaging studies, however, have not yet accounted for the potential role of close friendships in the association between peer rejection and social anxiety symptoms.

A final consideration when studying social affective neuroscience and psychopathology in adolescence is the role of pubertal status. Puberty is characterized by significant hormonal changes, with increases in secretion of the adrenal androgen dehydroepiandrosterone (DHEA) and gonadal hormones testosterone and estradiol (Crone & Dahl, 2012) that contribute to the physical growth and development of primary and secondary sexual characteristics seen during this period (Rogol, Roemmich, & Clark, 2002). Sex steroid hormones effect behavior at puberty through specific brain structures associated with socio-affective learning, including the amygdala (Romeo & Sisk, 2001).

Estradiol specifically has also been shown to affect prefrontal cortex function in young women (Jacobs & D'Esposito, 2011), while pubertal increases in testosterone have been linked to remodeling of neural circuits implicated in reward-seeking behaviors, including the nucleus accumbens (Blakemore, Burnett, & Dahl, 2010). DHEA, a precursor to both testosterone and estradiol, may exert similar effects on brain development during puberty, though these effects are poorly understood. Developmental models posit that an increase in pubertal sex hormones, and resulting changes in the brain, may contribute to the increased salience of social status during adolescence (Silk et al., 2014; Nelson et al., 2005; Blakemore, 2008). Interestingly, one study examining the neural correlates of peer rejection in adolescence found that, controlling for age, youth more advanced in adrenal signs of pubertal maturation compared to their peers showed increased reactivity to rejection in the bilateral amygdala, caudate, and sgACC, pointing to a potential role of DHEA in increasing sensitivity to rejection during adolescence (Silk et al., 2014).

Pubertal status is also important to consider given its association with social anxiety in adolescent girls. Not only are females more likely to develop social anxiety than males (Merikangas et al., 2010), but research has shown that only in girls is advanced pubertal development associated with higher levels of social anxiety (Kaltiala-Heino, 2003; Deardorff et al., 2007; Blumenthal et al., 2011). However, some studies have proposed complex explanations for how puberty relates specifically to the development of social anxiety. In a study with adolescents ages 10-17 years, Blumenthal et al. (2009) found no main effect of self-reported pubertal timing on social anxiety symptoms, however they did find that the combination of early pubertal development and negative peer relations (e.g., victimization) was positively associated with self-reported symptoms of social anxiety. Given the effects of pubertal maturation on function in social-affective neural regions and associations between puberty and social anxiety, the current

study examined the potential moderating role of puberty on the link between neural activation to peer rejection and social anxiety symptoms in an exploratory analysis.

1.5 The current study

There were two main goals of the current study. The first was to use ecologically valid measures to examine how neural sensitivity to peer rejection relates to affective responses to peer rejection in daily life, and how these two measures relate to current self-reported social anxiety symptoms in a sample of adolescent girls. The second goal was to examine how closeness to a best friend may moderate the links between neural sensitivity to rejection, real-world experiences of rejection, and social anxiety symptoms. An additional exploratory goal was to examine whether pubertal status also plays a role in understanding the link between neural sensitivity to rejection and current symptoms of social anxiety.

In pursuit of these goals, the current study employed both a neuroimaging task (Chatroom Interact Task) and a cell-phone Ecological Momentary Assessment (EMA) protocol to evaluate adolescent girls' brain responses to peer rejection in the laboratory and their self-reported affective responses to perceived peer rejection in daily life over a two-week period. These girls were oversampled for shy/fearful temperament, which places them at increased risk for developing social anxiety disorder (Chronis-Tuscano et al., 2009; Gladstone & Parker, 2006); thus, their peer relationships may be especially important.

There were five main hypotheses of the current study. The first hypothesis, attempting to replicate prior research (i.e., Rudolph et al., 2016), was that greater activation in a social-affective cortico-limbic network (including the amygdala, sgACC, dACC, and anterior insula) to peer

rejection would predict heightened, concurrent social anxiety symptoms in adolescent girls. The second hypothesis was that greater activation in this network would predict higher levels of social threat in daily life, measured using EMA. The third hypothesis was that social threat in daily life would be significantly associated with social anxiety symptoms. The fourth hypothesis was that social threat in daily life would explain the link between neural activation to rejection and social anxiety symptoms. Specifically, we hypothesized an indirect effect of real-world experiences of social threat on the relationship between neural activation to rejection and social anxiety symptoms in these girls, such that greater neural activation to rejection would predict a heightened affective response to real-world social threat, which in turn would predict greater social anxiety symptoms. The fifth hypothesis was that perceived closeness to a best friend would moderate the link between neural activation to peer rejection and social anxiety symptoms and/or the link between neural activation to peer rejection and social threat in daily life, such that adolescents with close friendships would show weaker associations between variables. An exploratory analysis also tested the hypothesis that girls more advanced in pubertal status would show a stronger association between neural activation to rejection and social anxiety symptoms.

2.0 Method

2.1 Participants

One-hundred-twenty-five adolescent girls ages 11 to 13 have been recruited for participation in a study on girls' brain development via advertisements and announcements in the community. A subsample of 99 girls (M_{age} = 12.30 years, SD = .83 years) was included in the current study, as recruitment was not completed by the time of data analysis for the current study. This subsample was predominately (66.7%) white. Total family income was used as a proxy for socioeconomic status (SES). Approximate total income over the past year was reported by participants' parents in increments of $10,000 on a scale of 0 ($0-10,000) to 10 ($100,000+). Mean total family income in this sample was a 6.65 (between $60-80,000) with a standard deviation of 3.37 and range of 0-10. Key demographic characteristics are summarized in Table 1. Girls in this study were oversampled for shy/fearful temperament, as this temperament in childhood has been shown to predict social anxiety in later adolescence and adulthood (Gladstone & Parker, 2006; Chronis-Tuscano et al., 2009). Two-thirds of this sample (n=66) was considered at "high-risk" for developing social anxiety and one-third (n=33) was considered at "low-risk" for developing social anxiety. Risk status was calculated using the Early Adolescent Temperament Questionnaire-Revised (EATQ- R; Ellis & Rothbart, 2001). Girls classified as "high-risk" had scores at least 0.75 standard deviations above the mean on the fear or shyness scales of either the parent- or child-reported EATQ-R, while girls classified as "low-risk" had scores < 0.75 standard deviations above the mean on both the fear and shyness scales of both parent- and child-reported EATQ-R.

To be eligible for the study, participants could not meet DSM-5 criteria for a current or lifetime diagnosis of any anxiety disorder (except for specific phobia), major depressive disorder (MDD), or any psychotic or autism spectrum disorder, as determined by the Kiddie-Schedule for Affective Disorders and Schizophrenia- Present and Lifetime version (K-SADS-PL; Kaufman, Birmaher, Brent, Rao, et al., 1997). All participants were right handed and had an IQ > 70 as assessed using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 2011). Additional exclusionary criteria include a lifetime presence of a neurological or serious medical condition, being pregnant (assessed at fMRI scan), the presence of any MRI contraindications (e.g., dental braces, any metal in the body, claustrophobia), uncorrected visual disturbance, presence of head injury or congenital neurological anomalies (based on parent report), acute suicidality, or taking medications that affect the central nervous system (e.g., SSRIs).

Table 1. Key demographic characteristics of the total sample

	n (%)	Mean (SD)	Range
Age		12.29 (.83)	11-13
Pubertal status			
Adrenal		3.39 (1.20)	1-5
Gonadal		3.63 (1.21)	1-5
Average score (Adrenal + Gonadal)		3.53 (1.07)	1-5
Total family income		6.65 (3.37)	0-10
Race/Ethnicity			
White	66 (66.7%)		
Black/African-American	24 (24.2%)		
Asian	1 (1.0%)		
Biracial	7 (7.1%)		
Other	1 (1.0%)		
Hispanic or Latino	6 (6.1%)		

Note. Pubertal status was coded as a continuous variable from 1 (low) to 5 (high); Total family income was reported on a scale of 0-10 in increments of $10,000 (e.g., 0=$0-10,000, 1=$10,001-20,000…10=$100,001+).

2.2 Procedure

The study was approved by the University of Pittsburgh Institutional Review Board. The study consisted of two visits to the lab (Visit 1 & Visit 2), one visit to the University of Pittsburgh Magnetic Resonance Research Center (MRRC; Visit 3), and a home protocol that took place in between Visits 2 and 3. Prior to Visit 1, parents completed the EATQ-R.

During their first visit to the lab (Visit 1), parents provided informed consent and youth provided informed assent to acknowledge their voluntary agreement to participate in the research. Following informed consent, a research assistant administered the WASI to each participant. In addition, the K-SADS-PL (parent and child interviews) was administered to each participant and her primary caregiver by a trained graduate student or doctoral level therapist. Using the K-SADS-PL, clinicians determined current and past DSM-5 diagnoses for each participant. At this visit, participants were asked to complete several questionnaires (see *Measures*), including the Screen for Anxiety and Related Emotional Disorders (SCARED) and Pubertal Development Scale (PDS).

During a follow-up visit to the lab (Visit 2), participants completed the first part of the Chatroom Interact Task (see Section 2.3.5). At the end of Visit 2, the participants were given an android smartphone to complete the EMA home protocol. Youth and their parents were given a tutorial on how to work the smartphone and provided with details about the EMA protocol.

Immediately following Visit 2, participants began the EMA home protocol using the smartphone that was provided for them. The EMA protocol lasted for 16 consecutive days (10 weekdays, 3 weekends). Participants were randomly sampled (within pre-specified blocks of time) three times on each weekday and four times on each weekend day. The protocol included questions about recent social interactions and emotional and behavioral responses to these reactions (see

Section 2.3.4). Participants were not scheduled to complete the EMA home protocol over holidays and vacations, and were rescheduled if they fell sick.

Approximately two weeks following Visit 2, youth visited the MRRC (Visit 3) to complete a functional magnetic resonance imaging (fMRI) brain scan. Prior to the start of the real fMRI scan, subjects completed a screening questionnaire and practiced the fMRI portion of the Chatroom Interact Task in a mock MRI scanner. Before beginning the real scan, subjects were told that they had been matched with two girls they rated highly at their second visit, and that they would be interacting with them online in real-time during the fMRI task (Chatroom Interact Task).

2.2.1 fMRI acquisition

Before entering the real scanner, participants were trained in a simulation MRI scanner ("mock scanner") to familiarize them with the tight space and the loud sounds of the scanner. Scanning took place on a 3T Siemens Prisma magnet. Task stimuli were projected onto a color, high-resolution LCD screen in front of the scanner bed and viewed in a mirror mounted on the head coil. Head movement was constrained by foam padding. Participants responded to stimuli using a handheld response glove. Functional scans were preceded by a localizer. Functional images were acquired using a multiband technique (with an acceleration factor of 3); each brain volume contains 60 contiguous 2.3-mm axial slices acquired parallel to the anterior commissure–posterior commissure line using a single-shot gradient echo with T2* weighting with the following parameters: 1500 ms repetition time (TR), 30 ms echo time (TE), $2 \times 2 \times 2$ mm voxels, 96×96 matrix and 220-mm field of view (FOV). A high-resolution anatomical image was also acquired during a six-minute high-resolution T1-weighted structural brain scan (magnetization prepared rapid acquisition gradient echo, MPRAGE). Following this scan, the Chatroom Interact Task was

administered in the scanner. Participants were equipped with a response glove on their right hand that allowed them to make responses during the task.

2.3 Measures

2.3.1 Instruments to assess eligibility

2.3.1.1 Risk status

Risk status was assessed using the Early Adolescent Temperament Questionnaire – Revised (EATQ-R; Ellis & Rothbart, 2001). The EATQ-R consists of 65 questions and 12 scales. The current study examined only the shyness and fear scales. The EATQ-R has identical child and parent (parent report on child) versions, except in the parent version the items are rephrased in terms of the parents' perspectives. For the current study, both adolescent self-report scores and parent-report scores on the EATQ-R shyness and fear scales were considered in the determination of risk status. In this sample, internal consistency for the EATQ-R shyness scale was moderate for adolescent self-report (Cronbach's $\alpha = .74$) and high for parent report ($\alpha = .87$). In this sample, internal consistency for the EATQ-R fear scale was low for adolescent self-report ($\alpha = .46$) and parent report ($\alpha = .66$).

The average score on the EATQ-R shyness scale for a healthy adolescent female is 2.88 (SD = 0.75). The average score EATQ-R fear scale for a healthy adolescent female is 2.80 (SD = 0.77; Muris & Meesters, 2009). In the current sample, the average score on the child-report shyness scale was a 2.79 (SD = .75, Range = 1 – 4.43), while the average score on the parent-report shyness scale was 2.74 (SD = .96, Range = 1 – 5.00). In the current sample, the average score on the child-

report fear scale was a 2.75 (SD = .68, Range = 1.17 – 4.50), while the average score on the parent-report fear scale was 2.33 (SD = .73, Range = 1 – 3.83).

For the current study, adolescents with a score > 0.75 SDs above the mean on the fear or shyness scales on either child or parent report were accepted into the study as part of the high-risk group. Adolescents with a score < 0.75 SDs above the mean on the fear or shyness scales of both child and parent reports were accepted into the study as part of the low-risk group.

Although prior research has found that parent- and child-reported scores on the fear scale (r = .40, p < .01) and shyness scale (r = .31, p < .05) correlate significantly for female adolescents (Ellis & Rothbart, 2001), only parent- and child-reported scores on the shyness scale correlated significantly in the current sample (r = .61, p < .001). They did not correlate significantly for the fear scale (r = .17, p = .099).

2.3.1.2 Diagnostic assessment

The Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997, updated for the DSM-5 in 2016), a structured diagnostic interview, was administered by a trained clinician to all participants before confirming their inclusion in the study. Participants were excluded from the current study if they received a diagnosis of a current or past anxiety disorder (except for specific phobia) or current or past major depressive disorder based on the K-SADS-PL. Participants were also excluded for any current or history of psychosis or autism spectrum disorder. The K-SADS-PL is a reliable and valid instrument for diagnosing anxiety disorders in children (Kaufman et al., 1997). The instrument has high interrater reliability (93-100%), good test-retest reliability (r = .77-1.00), and high concurrent validity, such that children who screened positive for any current anxiety disorder

scored significantly higher than other children on self-reported anxiety measures (Kaufman et al., 1997).

2.3.1.3 Brief intelligence measure

In addition to the K-SADS-PL, the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) was administered to each participant before confirming inclusion in the study. The WASI is a well-normed brief measure of general intelligence that includes both verbal and nonverbal tests. It has good concurrent validity, high internal consistency (Cronbach's $\alpha = .93$), and high test-retest stability (ICC = .95; McCrimmon & Smith, 2013). Participants with an IQ < 70 (suggestive of intellectual impairment) as determined by the WASI were excluded from the study.

2.3.2 Social anxiety symptomology

At Visit 1, participants completed a modified (44-item) version of the Screen for Anxiety and Related Emotional Disorders-Child version (SCARED; Birmaher et al., 1997). The original SCARED is a 41-item self-report checklist that assesses multiple symptoms of anxiety across several domains – generalized anxiety, social anxiety, school avoidance, panic symptoms, and separation anxiety. The modified version used in this study combines the 41-item SCARED with the social phobia subscale of the SCARED-71 (Bodden, Bogels, & Muris, 2009) to create a 44-item version with better coverage of social anxiety symptoms. The total SCARED and SCARED subscales have good internal consistency (Cronbach's $\alpha = .74$ to .93) and good test-retest reliability (ICCs = .80 to .90; Birmaher et al., 1997). The SCARED also correlates significantly with other anxiety symptom scales and successfully differentiates anxious from non-anxious subjects (area

under the curve, AUC = 0.97; Monga et al., 2000). For the current study, the social anxiety subscale (original social anxiety subscale plus the additional social phobia items from the SCARED-71) was used as a measure of social anxiety symptoms. Possible scores on this scale can range from 0 to 20. In the current sample, scores ranged from 0 to 11. Reliability of the social anxiety scale in this sample was high ($\alpha = .77$).

2.3.3 Pubertal status

At Visit 1, participants also completed the Female Pubertal Development Scale (PDS; Petersen et al., 1988), a self-report measure of physical development for youth under the age of 16. The PDS shows moderate reliability; in this sample, internal consistency was $\alpha = .71$. Correlations between the PDS and physician ratings range between .61 and .67 (Brooks-Gunn et al., 1987). Shirtcliff, Dahl, and Pollak (2009) developed a coding system to convert the PDS to a 5-point scale to parallel the physical exam Tanner stages. This coding system captures gonadal and adrenal hormonal signals of physical development. For girls, breast development, growth spurt, and menarche are associated with gonadal hormones, while pubic/body hair and skin changes are associated with adrenal hormones (Shirtcliff, Dahl, & Pollak, 2009). This coding system was used as a proxy of pubertal status in the current study, with three separate scores measuring distinct aspects of pubertal development: a gonadal score capturing changes associated with gonadal hormones (i.e., growth spurt, breast development, and menarche), an adrenal score capturing changes associated with adrenal hormones (i.e., skin changes and pubic hair), and an average score (i.e., average of the adrenal and gonadal scales).

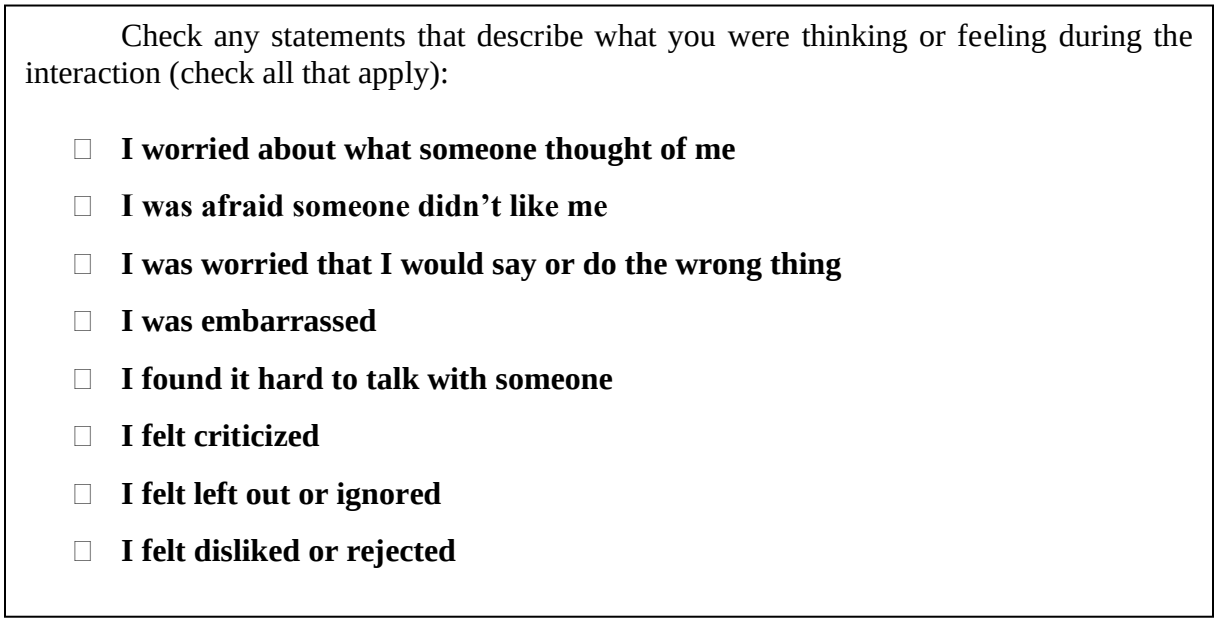
2.3.4 Ecological momentary assessment (EMA)

Data on real-world emotional responses to perceived experiences of peer rejection and subjective feelings of closeness/connectedness to a friend were collected using cell-phone EMA. Youth were given a pre-programmed android smartphone on which they entered their responses to a series of questions about their daily experiences with peers using a secure smartphone app for Web Data Express (WDX) developed by the Office of Academic Computing in the University of Pittsburgh Department of Psychiatry.

Using these cell-phones, participants were asked to answer questions about their most recent social interactions and their emotional and behavioral responses to these interactions for 16 consecutive days. On each of these 16 days, the adolescents were randomly sampled (i.e., received an electronic notification to respond) three times per day on weekdays (once in the morning between 7 AM and 8 AM and twice between 4 PM and 9:30 PM) and four times per day on the weekends between 10 AM and 9:30 PM, allowing for a maximum of 52 samples. This large number of samples allows for a more stable estimate of "typical functioning," even in the potential presence of several atypical days.

After receiving the electronic notification, adolescents were prompted through a series of questions about their recent moods and interactions with friends. Specifically, participants were first asked who they were with and how close/connected they felt to that person on a scale of 0-100. They were then asked to: "Think about the last interaction you had since the last beep that made you feel bad that you had with another kid your age." They were asked to type out (in a free response box) details about this interaction that occurred since the previous sampling and asked to indicate whether the interaction took place in person, over the phone, over text message, online, or on Facetime. In addition, participants were given a checklist that includes statements that

describe how they may have been thinking or feeling during the interaction (referred to as "social threat statements") and were asked to check off which statements applied to them in the situation (see Figure 1). Examples of social threat statements include, "I worried about what someone thought of me," and "I was afraid someone didn't like me." These questions took approximately 5 minutes to complete at each interval. A reliability analysis in SPSS v24.0 was conducted to confirm that all items (i.e., all social threat statements) loaded acceptably onto a single social threat factor. In the current study, reliability for these items was acceptable (α=.69). By collecting data in real-time and not retrospectively in the laboratory, EMA minimizes recall bias, maximizes ecological validity, and allows study of micro-processes that influence real-world behaviors (Shiffman, Stone, & Hufford, 2007).


Check any statements that describe what you were thinking or feeling during the interaction (check all that apply):

- ☐ **I worried about what someone thought of me**
- ☐ **I was afraid someone didn't like me**
- ☐ **I was worried that I would say or do the wrong thing**
- ☐ **I was embarrassed**
- ☐ **I found it hard to talk with someone**
- ☐ **I felt criticized**
- ☐ **I felt left out or ignored**
- ☐ **I felt disliked or rejected**

Figure 1. EMA social threat statements

2.3.5 Chatroom interact task

The Chatroom Interact Task is a mixed block/event-related functional magnetic resonance imaging (fMRI) task used to examine neural responses to peer rejection and acceptance (see Silk et al., 2012; 2014). This task is believed to be ecologically valid as it more closely mimics peer interactions in the form of an online chatroom. The first component of the Chatroom Interact Task was completed in the laboratory several weeks before the fMRI scan. During this laboratory visit, participants were shown photographs and biographical profiles for 20 same-sex, age-matched adolescents. Participants were told that these adolescents have previously participated in the study; however, these profiles were fictitious and were created by the researchers. Participants were asked to read the profiles and choose five peers with whom they would be most interested in interacting during the fMRI scan. To increase believability of, and investment in, the other peers, participants also provided their own photographs and created their own profiles to send to these peers.

When participants returned about two weeks later for the fMRI portion of the Chatroom Interact Task, they were told that they were matched with two of the adolescent girls they rated most highly at their first visit, and that these girls were also at their respective testing sites and preparing to interact with the participant online. During the fMRI portion of the task, pictures of the peers and participant were projected on a screen two at a time, and the participant and virtual peers took turns selecting who they would rather talk to about different potential interests (e.g., music, college). Unbeknownst to participants, the virtual peers' choices were pre-determined by the computer. The task is made up of four blocks with fifteen trials in each block, for a total run time of 15.1 minutes. During the first block, participants completed control trials, in which they indicated where a dot was placed on the screen (left or right). During the second block, participants chose which one of the virtual peers they would rather chat with. Analyses focused on the third

and fourth blocks, in which the subject is chosen/not chosen by their virtual peers. During "acceptance" trials, a virtual peer selects the participant to chat with. During "rejection" trials, a virtual peer selects the other peer to chat with, thus rejecting the participant. Following each selection, the photograph of the person who is chosen is highlighted, and the photograph of the person who is not chosen is superimposed with a large 'X'. To maintain task engagement, participants were asked to indicate, using a button press, whether the person on the left or the right was chosen when they are not the ones choosing.

2.4 Analytic plan

Using a series of path analyses conducted in Mplus (Muthén & Muthén, 2015; see Figure 2), the present study examined: 1) the relationships between neural activation to laboratory-based rejection, real-world experiences of social threat, and concurrent social anxiety symptoms, 2) the indirect effect of daily social threat on the relationship between neural activation to rejection and social anxiety symptoms, and 3) how friendship quality moderates the links between neural activation to rejection, real-world social threat experiences, and social anxiety symptoms. An exploratory analysis also examined how pubertal status moderates the link between neural sensitivity to rejection and social anxiety symptoms.

2.4.1 EMA data analysis

2.4.1.1 Social threat statements

The number of social threat statements that each participant checked off during each EMA sampling (52 maximum samplings) was summed and divided by the number of samplings to create an average social threat score. This analysis is sensitive to the fact that some participants did not respond to all EMA notifications or did not have a negative interaction with a peer every day, thus the number of total samplings differed across participants. This average social threat score was included as an observed variable, termed *social threat experience in daily life,* in the main model. If a participant did not endorse any negative interactions with peers over the 16 days of EMA data collection, her data was not used in the main analysis of the current study.

2.4.1.2 Friendship closeness

At each EMA sampling, participants were also asked who they were currently with (i.e., a friend, a sibling, a parent) and asked to rate how close/connected they felt to this person using a scale from 0-100. For each participant, these close/connected ratings were averaged using the ratings from each instance in which the participant reported being with a friend. Only participants who reported being with a friend $\geq$3 times over the 52 samples were included.

2.4.2 FMRI data preprocessing and analysis

All fMRI data were preprocessed according to standard protocols based on the general linear model (GLM), using a canonical hemodynamic response function, in SPM 12 (Wellcome Department of Cognitive Neurology, London, UK). The preprocessing procedure includes image

reconstruction and reorientation, coregistration with the high-resolution structural image, spatial realignment and normalization to a standard Montreal Neurological Institute (MNI) T1 template, and spatial smoothing using a 6 mm Gaussian kernel. High pass filtering (128 s) was applied to remove low frequency noise in the EPI signal. Head motion artifact was detected and ArtRepair was used to make appropriate adjustments. Any subjects with more than 25% of trials with excess movement were excluded (i.e., censored) from analyses. Using this threshold, data from 11 participants was deemed unusable and excluded from analyses.

For first-level analyses, regressors were computed for each individual for the two contrasts of interest (Peer Rejection>Peer Acceptance, Peer Rejection>Control). These two contrasts were chosen because the Control block is a new addition to the task, thus the reliability of this trial type is unknown. The long duration of each rejection trial allows for slow event-related analysis. Based on a priori hypotheses, a group-level region-of-interest (ROI) approach was then used. To conserve power, anatomically-defined ROI masks for the amygdala, dACC, sgACC, and anterior insula were pulled from WFU PickAtlas (http://fmri.wfubmc.edu/ software/pickatlas) and combined into one large "affective-salience" ROI mask. First-level contrasts for each participant were included in a second-level one-sample *t* test in SPM 12 to examine statistically significant activation within the ROI mask across participants. Eigenvariates, or estimates of signal intensity similar to a cluster's average signal, were extracted using the SPM VOI tool from clusters within this mask surviving a voxel-wise threshold of $p<.001$ uncorrected and cluster threshold of $p<.05$ family-wise-error (FWE) corrected with small volume correction (i.e., FWE correction was restricted to inside the mask). A secondary whole-brain analysis was also run to identify potential clusters outside of this affective-salience mask for both contrasts of interest (Reject>Accept,

Reject>Control). Eigenvariates were extracted from clusters surviving a voxel-wise threshold of $p<.001$ uncorrected and whole-brain cluster-wise threshold of $p<.05$ FWE corrected.

2.4.3 Main analyses

Extracted eigenvariates for each cluster were tested in a series of path analyses using structural equation modeling (SEM). These analyses were conducted in Mplus (Muthén & Muthén, 2015) using full information maximum likelihood (FIML) (Enders & Bandalos, 2001). Eigenvariates, scores from the social anxiety subscale of the SCARED (adolescent self-report), and social threat experience scores (EMA) were included as observed variables. In all models, age, race/ethnicity, and total family income (a proxy for socioeconomic status, SES) were included as covariates. Predictor variables were mean centered prior to analyses. Separate models were estimated for each significant cluster, with Benjamini-Hochberg procedures used to control for multiple tests with a false discovery rate of 0.10. This false discovery rate has been suggested for research in which the cost of a false positive is low and researchers do not want to miss anything that might be important (McDonald, 2014).

This general model was used to examine whether (i) heightened neural activation to peer rejection is associated with social anxiety symptoms (Hypothesis 1); (ii) heightened neural activation to peer rejection is associated with social threat scores (Hypothesis 2); (iii) heightened social threat in daily life is associated with symptoms of social anxiety (Hypothesis 3); (iv) there is an indirect effect of social threat in daily life on the link between heightened neural activation to peer rejection and social anxiety symptoms (Hypothesis 4); and (v) perceived closeness to a best friend moderates the links between neural response to peer rejection and social threat experience in daily life and/or between neural response to peer rejection and social anxiety symptoms in daily

life (Hypothesis 5). To test Hypothesis 1, the paths to and from social threat experience (Paths *a* and *b*) were set to 0, allowing the estimation of the total effect of neural reactivity on social anxiety symptoms (Path *c*). To test Hypothesis 2, the paths to social anxiety symptoms (Paths *b* and *c*) were set to 0, allowing the estimation of the effect of neural reactivity on social threat experience (Path *a*). To test Hypothesis 3, the paths from neural reactivity (Paths *a* and *c*) were set to 0, allowing the estimation of the effect of social threat experience on social anxiety symptoms (Path *b*). To test Hypothesis 4, all paths (Paths *a, b,* and *c*) were freely estimated and tests of mediation were implemented using the MODEL INDIRECT command in Mplus. This option computed specified indirect effects, including regression weights and standard errors. To test Hypothesis 5, friendship closeness was included as a moderator on Paths *a* and *c* in the full mediation model, allowing a test of conditional mediation (Figure 2). The MODEL CONSTRAINT command in Mplus was used to test the significance of the mediation at values of friendship closeness at the mean and 1 standard deviation above and below the mean.

Due to large amounts of missing data discovered post-hoc, the mediation model (Hypothesis 4) was re-run including only subjects with full data (n=57), as is done with listwise deletion, in follow-up analyses.

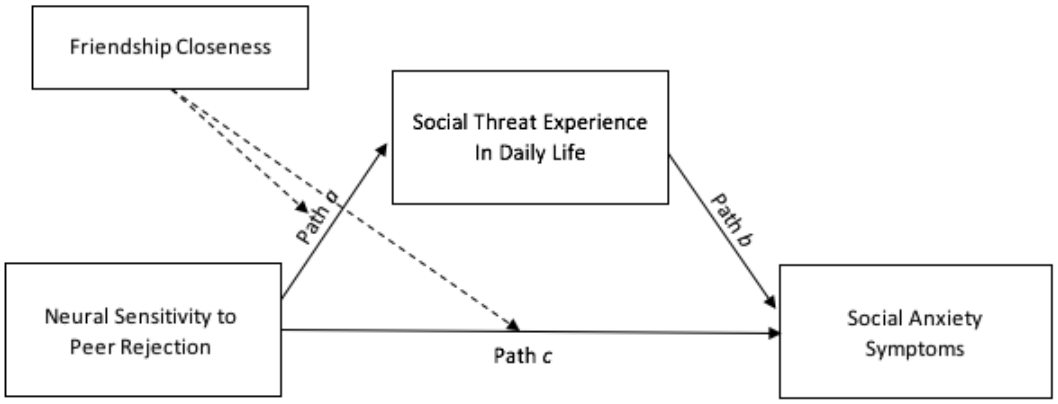


Figure 2. Conditional mediation model, with dotted lines representing moderation

2.4.4 Exploratory analyses

Exploratory analyses were run to examine a potential moderating role of pubertal status on the association between neural response to peer rejection and social anxiety symptoms. Each index of pubertal status (i.e., adrenal score, gonadal score, and average score) was included (in separate models) as a continuous variable (coded from 1 to 5) and set to moderate only the direct relationship between neural sensitivity and social anxiety symptoms. Girls with more advanced pubertal status on all indices were hypothesized to show a stronger association between neural sensitivity to peer rejection and social anxiety symptoms.

3.0 Results

3.1 Preliminary results

3.1.1 fMRI task

Of the 99 participants included in analyses, 62 had usable fMRI data. Of the 37 participants missing fMRI data, 14 had not yet had their scans completed, as data collection is still ongoing. Other reasons for exclusion included scan refusal (*n*=6), excess movement (*n*=11), subjects falling asleep in the scanner (*n*=4), incidental findings (*n*=1), and behavioral issues (i.e., subjects not responding behaviorally during the task) (*n*=1). Thus, 62 participants contributed fMRI data to the mediation model. These 62 participants did not differ from participants missing fMRI data (*n*=37) in age, race, SES, social anxiety scores, or social threat scores (*p*s>.122).

3.1.2 Social threat

The 8 social threat variables showed acceptable reliability (α=.69) for loading onto one social threat factor. When each social threat variable was summed across all the responses for each participant, reliability for the 8 variables increased (α=.82).

Eighty-nine participants completed the social threat questions using EMA at least one time over the two-week EMA collection period, thus 89 participants contributed social threat data to the mediation model. These 89 participants differed from participants who did not complete any social threat questions (*n*=10) on race (*p*=.047). Youth who completed the social threat questions

were predominately white (70.8%). Most participants (*n*=83) completed the social threat questions at least 3 times over the two-week period.

3.1.3 Friendship closeness

Sixty-three participants reported being with a friend during EMA collection at least 3 times over the two-week EMA collection period, thus 63 participants contributed friendship closeness data to the moderated mediation model. Youth who contributed closeness data differed from youth who did not contribute closeness data in SES (*p*=.033) and race (*p*=.007). Youth who contributed data were primarily white (77.8%) and had a mean total family income of 7.19 (out of 10), while youth who did not contribute data were more equally split in race (47.2% white, 44.4% black) and had a lower mean total family income of 5.69. Of note, the average friendship closeness variable was not significantly correlated with the average social threat variable (*r*=-.15, *p*=.243), thus multicollinearity in the conditional mediation model was not of concern.

3.1.4 Restricted data analysis

Of the 99 participants who contributed data to the current analyses, 57 had complete data, excluding friendship closeness scores (i.e., usable fMRI data, social threat data, and social anxiety scores). These 57 participants did not differ from participants missing fMRI data but included in the overall mediation model (*n*=42) in age, race, SES, social anxiety scores, or social threat scores (*p*s>.332).

3.2 FMRI results

Results from the one-sample *t*-test examining neural activation differences for both the Reject>Accept and Reject>Control contrasts in the affective-salience mask revealed no significant clusters within the mask for either contrast (*n*=62). However, a whole-brain analysis (one-sample *t*-test) revealed significant activation for the Reject>Accept contrast in one cluster in a region of the occipital lobe (Table 2). The whole-brain analysis for the Reject>Control contrast identified 11 clusters outside the affective-salience mask, including clusters in the caudate, precuneus, and angular gyrus (Table 2).

Table 2. Brain regions activated during the peer rejection condition relative to either the peer acceptance condition or a control block

Anatomical Region	BA	k	x	y	z	Z (peak)	$p_{FWE\text{-corrected}}$ (cluster-level)
Rejection>Acceptance							
Right Occipital Lobe Cortex	BA19	778	24	-76	-8	4.78	<.001
Rejection>Control							
Left Supramarginal Gyrus	BA40	3653	-52	-42	50	6.12	<.001
Left Premotor Cortex	BA6	1051	-26	8	62	5.68	<.001
Left Caudate	--	449	-2	12	12	5.29	<.001
Right Premotor Cortex	BA6	818	20	14	56	5.28	<.001
Right Angular Gyrus	BA39	407	36	-78	40	5.08	<.001
Left Primary Somatosensory Cortex	BA1	279	-58	18	14	4.97	.002
Left Fusiform Gyrus	BA37	437	-56	-58	-6	4.95	<.001
Right Precuneus	BA7	640	12	-60	66	4.89	<.001
Right Middle Temporal Gyrus	BA21	307	64	-46	6	4.79	.001
Right Supramarginal Gyrus	BA40	355	46	-24	22	4.36	<.001

Note. BA = Brodmann area; k = cluster size; x,y,z = MNI coordinates.

3.3 Path analyses

Descriptive statistics and intercorrelations between variables included in the final mediation model can be found in Table 3. Small to moderate correlations were found between total family income (a proxy for SES) and social anxiety symptoms (r=-.22) and between age and social threat in daily life (r=.16). Differences in neural activation by race were also seen for some brain regions, including the left caudate. Thus, total family income, age, and race were controlled for in all analyses.

Examining the direct effect of neural activation on social anxiety scores (Hypothesis 1; Path *c*), controlling for age, race, and SES, we found no significant associations between activation in any region and social anxiety. Examining the effect of neural activation on social threat scores (Hypothesis 2; Path *a*), controlling for age, race, and SES, revealed that only activation in the caudate nucleus cluster for the Reject>Control contrast (Figure 3) significantly predicted social threat scores (β=.38, SE=.12, uncorrected p=.001, Benjamini-Hochberg p=.020). No outliers were detected. Examining the effect of social threat scores on social anxiety scores (Hypothesis 3; Path *b*), controlling for age, race, and SES, revealed a significant effect of social threat on social anxiety (β=.24, SE=.10, p=.015) as well as a significant effect of SES on social anxiety (β= -.26, SE=.10, p=.011).

The full mediation model was tested using activation values from the caudate cluster for Reject>Control, as this was the only cluster that was significantly associated with either social threat scores or social anxiety scores. The model was conducted using FIML with 99 subjects, controlling for age, race, and SES, with bootstrapped standard errors (bootstrapping = 10,000). In this model, the indirect effect of social threat scores on the link between caudate activation and social anxiety was significant, given that the confidence interval did not contain zero (B=.50,

SE=.32, 95% CI [.04, 1.34]). Caudate activation was significantly associated with social threat scores (Path *a*; β=.38, B=.53, SE=.17, *p*=.001, 95% CI [.18, .83]) and social threat scores were associated with social anxiety scores at trend level (Path *b*; β=.25, B=.94, SE=.50, *p*=.060, 95% CI [-.06, 1.89]). A significant effect of SES on social anxiety also emerged in this model (β=-.23, B=-.16, SE=.08, *p*=.048, 95% CI [-.33, -.006]). No significant direct effect of caudate activation on social anxiety scores (B=.08, SE=.70, 95% CI [-1.35, 1.38]) or total effect (B=.58, SE=.62, *p*=.350, 95% CI [-.65, 1.79]) emerged. A summary of model results can be found in Table 4.

Table 3. Descriptive statistics and correlations between variables included in full mediation and moderated mediation models

	Social anxiety	Social threat	Caudate	Closeness	Pubertal status	Total income	Age
Social anxiety	1						
Social threat	0.22	1					
Caudate	0.12	0.36	1				
Closeness	-0.05	-0.15	-0.19	1			
Pubertal status	-0.09	-0.06	-0.00	-0.15	1		
Total income	-0.21	0.05	-0.04	0.00	-0.14	1	
Age	-0.10	0.16	0.07	0.04	0.48	0.16	1
MEAN	3.00	0.68	0.37	75.89	3.53	6.93	12.29
STDEV	2.36	0.62	0.46	17.05	1.07	3.37	0.83
RANGE	0-11	0-2.25	-.85-1.40	40-100	1-5	0-10	11-13

Note. Race is not included in this table because it is a categorical variable; Caudate = caudate activation to peer rejection relative to control; Closeness = close/connectedness EMA variable; Social threat = social threat experience in daily life EMA variable; Total income was rated on a 0-10 scale in increments of $10,000.

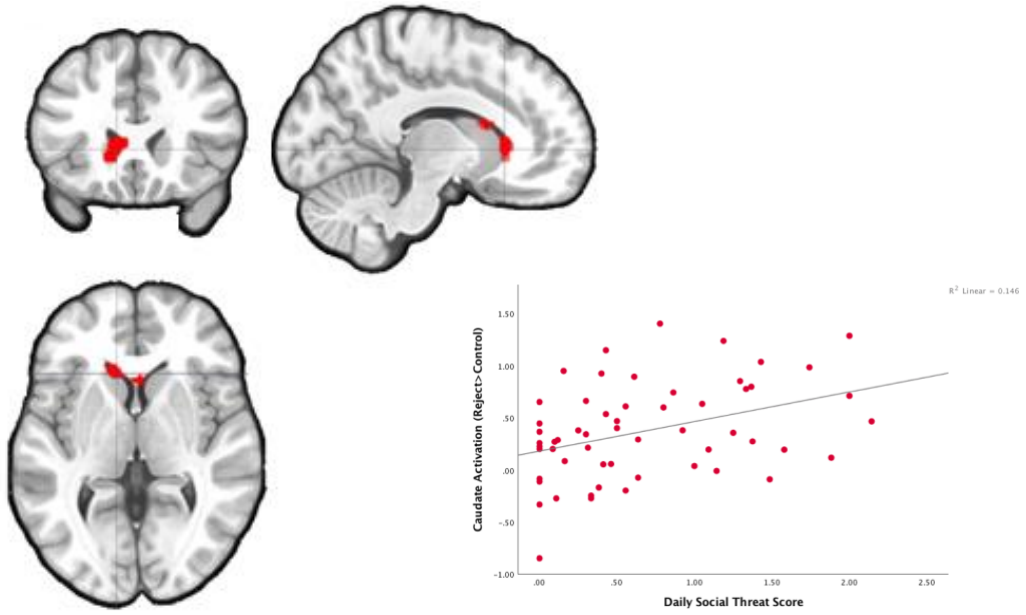


Figure 3. Activation in the left caudate nucleus cluster (left) resulting from the whole-brain results for the Peer Rejection > Control contrast correlated significantly with daily experiences of social threat (right)

Table 4. Summary of mediation model (*n*=99)

	β	B	SE	*p*-value	95% CI
Caudate activation to social anxiety symptoms (Path *c*)	.02	.08	.70	.905	-1.35 – 1.38
Caudate activation to social threat (Path *a*)	.38	.53	.17	.001	.19 – .83
Social threat to social anxiety symptoms (Path *b*)	.25	.94	.50	.060	-.06 – 1.89
Total Effect		.58	.62	.350	-.65 – 1.79
Total Indirect Effect		.50	.32	.124	.04 – 1.34
Total Direct Effect		.08	.70	.905	-1.35 – 1.38

Note. Covariates (age, race, SES) were included in analyses but are not included in the table for simplification; *β* = standardized beta; B = unstandardized beta; SE = standard error.

3.3.1 Restricted path analyses

Including only subjects with complete data (*n*=57), the indirect effect of social threat on the link between caudate activation to rejection and social anxiety symptoms became stronger (B=.83, SE=.39, 95% CI [.08, 1.59]). In this model, the effect of social threat on social anxiety was significant (β=.43, B=1.65, SE=.57, *p*=.004, 95% CI [0.54, 2.76]), as well as the effect of caudate activation to rejection on social threat (β=.38, B=.51, SE=.15, *p*=.001, 95% CI [0.20, 0.81]). No significant direct effect of caudate activation on social anxiety scores (B=.23, SE=.68, 95% CI [-1.09, 1.56]) or total effect (B=1.07, SE=.68, 95% CI [-.29, 2.43]) emerged.

3.3.2 Effects of friendship closeness

In the full moderated mediation model (i.e., conditional mediation model) tested in Mplus, friendship closeness did not significantly moderate the association between caudate activation to rejection and social anxiety symptoms (Moderation of Path *c*; B=-.61, SE=1.14, *p*=.593, 95% CI [-3.05, 1.50]) or the association between caudate activation to rejection and social threat in daily life (Moderation of Path *a*; B=-.01, SE=.34, *p*=.989, 95% CI [-.73, .61). A summary of results from the conditional mediation model can be found in Table 5.

Table 5. Summary of conditional mediation model

	B	SE	*p*-value
Caudate activation to social anxiety symptoms (Path *c*)	-.32	.96	.743
Friendship closeness to social anxiety symptoms	.39	.50	.442
Caudate activation X friendship closeness to social anxiety symptoms (i.e., moderation of Path *c* by closeness)	-.61	1.14	.593
Caudate activation to social threat (Path *a*)	.52	.25	.038
Friendship closeness to social threat	-.16	.17	.357
Caudate activation X friendship closeness to social threat (i.e., moderation of Path *a* by closeness)	-.01	.34	.989
Social threat to social anxiety symptoms (Path *b*)	1.97	.64	.002

Note. Covariates (age, race, SES) were included in analyses but are not included in the table for simplification; Betas are unstandardized; SE = standard error.

3.3.3 Effects of pubertal status

Neither the adrenal score, gonadal score, nor the average of both scores emerged as a significant moderator on the link between neural activation to rejection and social anxiety scores for any of the 12 clusters (*p*s>0.05).

4.0 Discussion

Findings from the current study support a link between neural activation to peer rejection in the laboratory and perceptions of social threat in daily life. Specifically, we found that activation in the caudate nucleus to peer rejection relative to a control was significantly associated with perceived social threat in daily life, measured using EMA, in a sample of early adolescent females. Contrary to hypotheses, we did not find that activation in this region, or any other brain region, to peer rejection was directly associated with social anxiety symptoms. However, we did find support for a small indirect effect of social threat in daily life on the link between caudate activation to rejection and social anxiety symptoms. In line with modern approaches, the presence of an indirect effect can be found absent a significant direct effect or total effect (Hayes, 2018), especially with small sample sizes (Rucker et al., 2011). This finding suggests that adolescent females with higher caudate nucleus activation to rejection report more social threat in daily life, which in turn is associated with higher symptoms of social anxiety. When restricting analyses to only participants with complete data, we found a stronger indirect effect of social threat in daily life on the link between caudate activation and social anxiety symptoms. Including more participants with full data will be important to further confirm (or deny) this finding.

Aligning with prior work showing that adults with greater activation in brain regions that process social threat report greater social distress in daily life (Eisenberger et al., 2007), we found that adolescent girls with greater activation in the caudate nucleus to peer rejection (vs. a control block) reported more negative thoughts and feelings associated with social threat in daily life. The caudate nucleus has been found to respond to both negative and positive feedback during learning and may moderate the influence of social feedback on social learning (Tricomi et al., 2006).

Reinforcement learning models posit that the caudate plays the "actor" role in the "actor-critic" model, while the ventral striatum plays the role of critic (Joel, Niv, & Ruppin, 2002). The critic uses a temporal difference prediction error signal to update predictions of future reward, while the actor uses a similar signal to modify stimulus-response associations to guide behavior so that rewards are chosen more frequently in the future. Based on this interpretation, the caudate plays more of a role in reward-based, and potentially punishment-based, learning guided by the predictive behavior of the ventral striatum. While this is supported by some work (e.g., O'Doherty et al., 2004), other studies challenge this view, and suggest that the caudate plays more of a predictive and evaluative role than the actor-critic model would suggest (Asaad & Eskandar, 2011). Interestingly, the caudate is also believed to play a role in guiding goal-directed behaviors in a social context. Greater caudate activity during rewards and punishments has been linked to greater changes in future behavior during a social exchange task (King-Casas et al., 2005), implicating the caudate again in the evaluation of action-outcome contingencies underlying goal-directed behavior (Grahn, Parkinson, & Owen, 2008).

Although the Chatroom task is not by design a learning task, one interpretation of the current findings is that youth with greater caudate activation to peer rejection in the laboratory are also more likely to attend to and recall peer rejection and social threat in daily life, potentially with the ultimate goal of using this information to alter their future behavior so that they are more likely to be accepted. This increased attention to social threat in the environment may place youth at higher risk for social anxiety symptoms, which could explain the indirect effect of social threat in daily life on the link between caudate activation to rejection and social anxiety symptoms. Importantly, the relationship between caudate activation to rejection and social threat in daily life is likely bidirectional, such that higher caudate activation to rejection may contribute to greater

perception of social threat in daily life, while more experiences of social threat in daily life may also influence caudate activity in response to social rejection. Thus, high caudate activation to rejection might also serve as a marker of greater social threat in daily life. This bidirectional relationship may be particularly important during adolescence, when significant changes in brain maturation and social contexts co-occur and likely have important influences on each other (Blakemore & Mills, 2014). Longitudinal work will help tease out how these brain-behavior associations play out across development.

Although not selected as one of our a priori regions of interest, the caudate has been implicated in processing social rejection in several prior studies (e.g., Slavich et al., 2010; Masten et al., 2009; Eisenberger et al., 2007; Gunther Moor et al., 2010; Cacioppo et al., 2013). A recent review found that while reliving rejection by a significant other, the caudate nucleus is reliably recruited in adults (Cacioppo et al., 2013), supporting an important role of the caudate in processing salient social rejection. Further, Slavich et al. (2010) found that greater caudate activity during social exclusion (vs. inclusion) was significantly associated with greater inflammatory responses to the Trier Social Tress Test (TSST) in a sample of adults, suggesting that individuals with more caudate activation to social exclusion are also more reactive to social stress in a real-world context, consistent with current findings. Also consistent with current findings, Eisenberger et al. (2007) found that adults with greater activation in the caudate to social exclusion (vs. inclusion) reported lower daily social support measured using EMA. Finally, Gunther Moor et al. (2010) found increased activation in the left caudate following rejection (vs. acceptance) when adults expected to be negatively evaluated (Gunther Moor et al., 2010). Aligning with our current interpretation, individuals with greater caudate activation to rejection might also be more likely to attend to, recall, and expect negative evaluation and social threat in daily life, which could reflect

a history of frequent negative evaluation. Importantly, the current study extends these prior studies in adults to a sample of adolescent females. Despite the fact that the caudate was reported in the results of these studies, the role of the caudate in social rejection processing is rarely interpreted or discussed. Given that this finding has now been replicated several times, more work is needed to determine the specific role of the caudate in social exclusion.

Current findings were restricted to the left caudate. While this could represent meaningful left-lateralization, prior research implicating the caudate in social rejection is mixed, with some research finding evidence of only left caudate involvement in processing rejection (Eisenberger et al., 2007; Gunther Moor et al., 2010) and some finding evidence of only right caudate involvement (Slavich et al., 2011; Masten et al., 2009; Cacioppo et al., 2013). Interestingly, however, one study found left-lateralized caudate metabolic abnormalities in adolescents with major depressive disorder (Gabbay et al., 2007), which may suggest associations between left caudate abnormalities and depression. This may be especially relevant in the current study, as participants were recruited based on a shy/fearful temperament, which may place them at risk for depression (Compas, Connor-Smith, & Haser, 2004). Future longitudinal measures obtained in the current study will be able to test whether girls with heightened caudate activation to rejection are at greater risk for the development of depression.

In this study we also found initial support for the reliability of novel EMA measures of social threat in daily life. Additionally, we found that adolescent girls who reported feeling more socially threatened with peers in daily life also reported more symptoms of social anxiety. This association is likely bidirectional, such that negative experiences with peers contribute to greater social anxiety, and youth with more social anxiety report more negative experiences with peers (Siegel, La Greca, & Harrison, 2009).

Surprisingly, in contrast to prior research, we did not find heightened activation to peer rejection (versus acceptance or control) in any regions within our affective-salience mask (i.e., dACC, sgACC, anterior insula, and amygdala). This may reflect methodological factors. In the current study, the anticipation of social feedback was modeled with feedback outcome (rejection or acceptance). Thus, some regions within the affective-salience mask might have been more active during the anticipation phase, while others may have been more active during the outcome phase. We could not isolate these differences in current analyses, which may have obscured some results. This modeling approach may also help explain why we did not see increased activation in any region except the occipital lobe to peer rejection relative to peer acceptance, which is the contrast typically used in similar work (e.g., Guyer et al., 2008; Rudolph et al., 2016).

Because we did not find heightened activation to peer rejection in any region inside the affective-salience mask, we were unable to test associations between activation in these regions and social anxiety symptoms. Thus, we were unable to replicate prior research showing that greater activation in these regions (i.e., dACC, sgACC, insula, amygdala) during peer exclusion relative to inclusion predicted social anxiety symptoms or diagnosis (Guyer et al., 2008; Rudolph et al., 2016). Interestingly, we also did not find associations between activation in *any* brain region to peer rejection (relative to peer acceptance or a control) and social anxiety symptoms. This is especially notable given that we found a significant indirect effect of daily social threat on the link between caudate activation to rejection and social anxiety symptoms absent a significant direct effect of caudate activation on social anxiety symptoms. There are several potential explanations for why we might detect an indirect effect in the absence of a direct effect. First, we may have been underpowered to detect a direct effect because of a small sample size or because of issues with measurement precision (Rucker et al., 2011), although this latter option is unlikely given that

the standard errors for caudate activation to rejection and social threat in daily life were comparable (.058 and .066, respectively). The second potential explanation is that the independent variable (caudate activation to rejection) is more strongly associated with the mediator (social threat in daily life) than the outcome variable (social anxiety symptoms). As discussed previously, this aligns with prior work showing that neural responsivity to peer rejection is associated with rejection sensitivity (Burklund et al., 2007; Masten et al., 2009). Finally, the presence of an unmeasured opposing indirect effect may have concealed a significant direct or total effect (Rucker et al., 2011). Furthermore, the relationship between caudate activation to rejection and social anxiety might involve not only the indirect effect of social threat, but also the indirect effect of a fourth suppressing variable. Omission of this suppressor would lead to total and direct effects that appear small and insignificant (Rucker et al., 2011). This suggests that examining multiple, potentially competing, indirect effects could enhance understanding of how neural activation to peer rejection is associated with social anxiety.

Inconsistent with our hypotheses, we also did not find that pubertal status moderated the link between neural activation to rejection and social anxiety symptoms. This was particularly surprising given prior work showing that healthy youth more advanced in pubertal status showed increased reactivity to rejection in the caudate (Silk et al., 2014). This may be attributable, in part, to the self-report measure of puberty used in the current study, and future studies may benefit from using more objective and complementary measures of pubertal status, including pubertal hormone levels. Further, we did not find that friendship closeness moderated the link between caudate activation to rejection and social threat or the link between caudate activation and social anxiety. We may have been underpowered in these analyses, as only 63 participants contributed friendship closeness data and only 41 of these 63 had neuroimaging data. In addition, our measure of

friendship closeness relied on child self-report through EMA and in some cases the measure was averaged from only three or four interactions that a participant had with a friend, which likely introduced significant variability. More objective measures of friendship closeness or more samplings of closeness over a longer time period will be important to consider moving forward.

4.1 Limitations and future directions

The current study benefits from a large sample and more ecologically-valid measures, yet there are a few important limitations. First, we were missing a significant amount of fMRI data. Although FIML is seen as a reliable method for estimating the population parameters from the available sample data (Enders & Bandalos, 2001), there has been little discussion in the field regarding whether FIML can reliably estimate missing fMRI data specifically, given the significant variability and instability inherent in fMRI data. However, researchers have commented that multiple imputation seems to be a reasonable approach for dealing with missing fMRI data (Vaden Jr. et al., 2012). Of note, FIML has been used in models with fMRI data in recent research (e.g., Swartz et al., 2017; Gard, Shaw, Forbes, & Hyde, 2018), with little concern expressed regarding its reliability. The second limitation is that this study is cross-sectional, which limits us from testing true mediation and understanding the potential developmental processes through which brain function and social threat influence social anxiety. Notably, however, the data presented in this paper is part of a larger longitudinal study, and neuroimaging, EMA, and questionnaire data is currently being collected at ages 13-15 (2-year-follow-up) and 14-16 (3-year-follow-up). Thus, future work will be able to test longitudinally how heightened neural activity to rejection at ages 11-13 predicts social threat experiences at ages 13-15 and social anxiety symptoms at ages 14-16.

Third, this study relies largely on adolescent self-report of social threat experiences and social anxiety symptoms, though the inclusion of daily EMA provides an improvement over traditional retrospective questionnaire reporting. Future work should examine whether findings hold using clinician-rated or parent-rated symptoms. Fourth, as discussed above, the task modeled both the anticipation and receipt of feedback (rejection, acceptance) together, which may help explain the absence of significant positive activation to peer rejection versus acceptance or control in our original regions of interest (i.e., amygdala, insula, ACC). Future work examining the neural correlates of anticipation and receipt of feedback separately may speak to this potential issue. Finally, this sample included only adolescent females ages 11 to 13, thus results may not generalize to older adolescent females or adolescent males.

5.0 Conclusions

Overall, this study is novel in its approach to linking fMRI and EMA measures to better understand how peer rejection may be linked to social anxiety symptoms in adolescent girls. Findings suggest that girls with heightened activation to peer rejection in the caudate nucleus, a brain region involved in social feedback learning and goal-directed behavior, report more social threat in daily life, which in turn is associated with higher social anxiety symptoms. This may suggest that adolescent girls with higher caudate activity are more likely to attend to and recall social threat in their environments, which places them at greatest risk for social anxiety symptoms. Heightened caudate activation to peer rejection may also reflect a history of negative social interactions that makes youth more perceptive to future social threat and increases risk for social anxiety. Although in this study we were interested in what might explain the link between neural processing of rejection and social anxiety symptoms, this model could in theory be tested with neural activity as the mediating variable. This latter option could speak to potential neural mechanisms that explain the link between peer rejection and social anxiety symptoms, and is an interesting question that should be followed up in future work. The reliability of our EMA social threat measure, along with the strong relationship between social threat in daily life and social anxiety symptoms, inspires confidence in this novel EMA measure of social threat. Data collection for this study is ongoing; thus, these analyses will be recomputed with a larger sample and more complete dataset. In addition, future work will examine associations between social threat, neural reactivity, and social anxiety longitudinally to test whether neural reactivity to peer rejection and social threat in daily life places adolescent females at higher risk for developing social anxiety

disorder. This continued work is important for understanding how brain-behavior associations impact the development of social anxiety disorder at a sensitive period of development.

Bibliography

Asaad, W. F., & Eskandar, E. N. (2011). Encoding of both positive and negative reward prediction errors by neurons of the primate lateral prefrontal cortex and caudate nucleus. *Journal of Neuroscience*, *31*(49), 17772-17787. https://doi.org/10.1523/JNEUROSCI.3793-11.2011

Birmaher, B., Khetarpal, S., Brent, D.A., Cully, M., Balach, L., Kaufman, J., Neer, S.M. (1997). The Screen for Child Anxiety Related Emotional Disorders (SCARED): Scale construction and psychometric characteristics. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(4), 545-553. https://doi.org/10.1097/00004583-199704000-00018

Blakemore, S. J. (2008). The social brain in adolescence. *Nature Reviews: Neuroscience*, *9*(4), 267-277. https://doi.org/10.1038/nrn2353

Blakemore, S. J., Burnett, S., & Dahl, R. E. (2010). The role of puberty in the developing adolescent brain. *Human Brain Mapping*, *31*(6), 926-933. https://doi.org/10.1002/hbm.21052

Blakemore, S-J., Mills, K.L. (2014). Is adolescence a sensitive period for sociocultural processing? *Annual Review of Psychology, 65*, 187-207. https://doi.org/10.1146/annurev-psych-010213-115202

Blumenthal, H., Leen-Feldner, E. W., Babson, K. A., Gahr, J. L., Trainor, C. D., & Frala, J. L. (2011). Elevated social anxiety among early maturing girls. *Developmental Psychology, 47*(4), 1133. https://doi.org/10.1037/a0024008

Blumenthal, H., Leen-Feldner, E. W., Trainor, C. D., Babson, K. A., & Bunaciu, L. (2009). Interactive roles of pubertal timing and peer relations in predicting social anxiety symptoms among youth. *Journal of Adolescent Health*, *44*(4), 401-403. https://doi.org/10.1016/j.jadohealth.2008.08.023

Bodden, D. H., Bögels, S. M., & Muris, P. (2009). The diagnostic utility of the screen for child anxiety related emotional disorders-71 (SCARED-71). *Behaviour Research and Therapy*, *47*(5), 418-425. https://doi.org/10.1016/j.brat.2009.01.015

Bolling, D.Z., Pitskel, N.B., Deen, B., Crowley, M.J., Mayes, L.C., Pelphrey, K.A. (2011). Development of neural systems for processing social exclusion from childhood to adolescence. *Developmental Science, 14*, 1431–44. https://doi.org/10.1111/j.1467-7687.2011.01087.x

Brooks-Gunn, J., Warren, M. P., Rosso, J., & Gargiulo, J. (1987). Validity of self-report measures of girls' pubertal status. *Child Development*, 829-841. https://doi.org/10.2307/1130220

Burklund, L. J., Eisenberger, N. I., & Lieberman, M. D. (2007). The face of rejection: rejection sensitivity moderates dorsal anterior cingulate activity to disapproving facial expressions. *Social Neuroscience, 2*(3-4), 238-253. https://doi.org/10.1080/17470910701391711

Cacioppo, S., Frum, C., Asp, E., Weiss, R. M., Lewis, J. W., & Cacioppo, J. T. (2013). A quantitative meta-analysis of functional imaging studies of social rejection. *Scientific Reports, 3*, 2027. https://doi.org/10.1038/srep02027

Chronis-Tuscano, A., Degnan, K.A., Pine, D., Perez-Edgar, K., Henderson, H.A., Diaz, Y., Raggi, V.L., Fox, N.A. (2009). Stable early maternal report of behavioral inhibition predicts lifetime Social Anxiety Disorder in adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry, 48,* 928-935. https://doi.org/10.1097/CHI.0b013e3181ae09df

Cohen, S., Wills, T. A. (1985). Stress, social support, and the buffering hypothesis. *Psychological Bulletin, 98,* 310–357. https://doi.org/10.1037/0033-2909.98.2.310

Compas, B. E., Connor-Smith, J., & Jaser, S. S. (2004). Temperament, stress reactivity, and coping: Implications for depression in childhood and adolescence. *Journal of Clinical Child and Adolescent Psychology, 33*(1), 21-31. https://doi.org/10.1207/S15374424JCCP3301_3

Crone, E. A., & Dahl, R. E. (2012). Understanding adolescence as a period of social-affective engagement and goal flexibility. *Nature reviews. Neuroscience, 13*(9), 636-650. https://doi.org/10.1038/nrn3313

Deardorff, J., Hayward, C., Wilson, K. A., Bryson, S., Hammer, L. D., & Agras, S. (2007). Puberty and gender interact to predict social anxiety symptoms in early adolescence. *Journal of Adolescent Health, 41*(1), 102-104. https://doi.org/10.1016/j.jadohealth.2007.02.013

Eisenberger, N. I., Gable, S. L., & Lieberman, M. D. (2007). Functional magnetic resonance imaging responses relate to differences in real-world social experience. *Emotion, 7*(4), 745-754. https://doi.org/10.1037/1528-3542.7.4.745

Eisenberger, N.I., Lieberman, M.D., Williams, K.D. (2003). Does rejection hurt? An fMRI study of social exclusion. *Science, 302*(5643), 290–292. https://doi.org/10.1126/science.1089134

Enders, C. K., & Bandalos, D. L. (2001). The relative performance of full information maximum likelihood estimation for missing data in structural equation models. *Structural equation modeling, 8*(3), 430-457. https://doi.org/10.1207/s15328007sem0803_5

Erath, S. A., Flanagan, K. S., & Bierman, K. L. (2007). Social anxiety and peer relations in early adolescence: Behavioral and cognitive factors. *Journal of Abnormal Child Psychology, 35*(3), 405-416. https://doi.org/10.1007/s10802-007-9099-2

Essau, C. A., Conradt, J., & Petermann, F. (1999). Frequency and comorbidity of social phobia and social fears in adolescents. *Behaviour Research and Therapy, 37*(9), 831-843. https://doi.org/10.1016/s0005-7967(98)00179-x

Festa, C.C., Ginsburg, G.S. (2011). Parental and peer predictors of social anxiety in youth. *Child Psychiatry Hum Dev, 42*(3), 291-306. https://doi.org/10.1007/s10578-011-0215-8

Fischer, S., Breithaupt, L., Wonderlich, J., Westwater, M. L., Crosby, R. D., Engel, S. G., ... & Wonderlich, S. (2017). Impact of the neural correlates of stress and cue reactivity on stress related binge eating in the natural environment. *Journal of Psychiatric Research, 92*, 15-23. https://doi.org/10.1016/j.jpsychires.2017.03.017

Forbes, E. E., Hariri, A. R., Martin, S. L., Silk, J. S., Moyles, D. L., Fisher, P. M., ... & Dahl, R. E. (2009). Altered striatal activation predicting real-world positive affect in adolescent major depressive disorder. *American Journal of Psychiatry*, *166*(1), 64-73. https://doi.org/10.1176/appi.ajp.2008.07081336

Gabbay, V., Hess, D. A., Liu, S., Babb, J. S., Klein, R. G., & Gonen, O. (2007). Lateralized caudate metabolic abnormalities in adolescent major depressive disorder: a proton MR spectroscopy study. *American Journal of Psychiatry, 164*(12), 1881-1889. https://doi.org/10.1176/appi.ajp.2007.06122032

Gard, A. M., Shaw, D. S., Forbes, E. E., & Hyde, L. W. (2018). Amygdala reactivity as a marker of differential susceptibility to socioeconomic resources during early adulthood. *Developmental Psychology, 54*(12), 2341-2355. https://doi.org/10.1037/dev0000600

Ginsburg, G.S., La Greca, A.M., Silverman, W.K. (1998). Social anxiety in children with anxiety disorders: relation with social and emotional functioning. *J Abnorm Child Psychol, 26*(3), 175-85.

Gladstone, G.L. & Parker, G.B. (2006). Is behavioral inhibition a risk factor for depression? *Journal of Affective Disorders, 95*, 85-94. https://doi.org/10.1016/j.jad.2006.04.015

Grahn, J. A., Parkinson, J. A., & Owen, A. M. (2008). The cognitive functions of the caudate nucleus. *Progress in Neurobiology, 86*(3), 141-155. https://doi.org/10.1016/j.pneurobio.2008.09.004

Gunther Moor, B., van Leijenhorst, L., Rombouts, S.A., Crone, E.A., Van der Molen, M.W. (2010). Do you like me? Neural correlates of social evaluation and developmental trajectories. *Social Neuroscience, 5*, 461–482. https://doi.org/10.1080/17470910903526155

Guyer, A.E., Choate, V.R., Pine, D.S., Nelson, E.E. (2012). Neural circuitry underlying affective response to peer feedback in adolescence. *Social Cognitive and Affective Neuroscience, 7*(1), 81-92. https://doi.org/10.1093/scan/nsr043

Guyer, A.E., Lau, J.Y.F., McClure-Tone, E.B., Parrish, J., Shiffrin, N.D., Reynolds, R.C., Chen, G., Blair, R.J.R., Leibenluft, E., Fox, N.A., Ernst, M., Pine, D.S., Nelson, E.E. (2008).

Amygdala and ventrolateral prefrontal cortex function during anticipated peer evaluation in pediatric social anxiety. *Arch Gen Psychiatry, 65*(11), 1303-1312. https://doi.org/10.1001/archpsyc.65.11.1303

Guyer, A.E., McClure-Tone, E.B., Shiffrin, N.D., Pine, D.S., Nelson, E.E. (2009). Probing the neural correlates of anticipated peer evaluation in adolescence. *Child Development, 80*(4), 1000–15. https://doi.org/10.1111/j.1467-8624.2009.01313.x

Hasson, U., & Honey, C. J. (2012). Future trends in Neuroimaging: Neural processes as expressed within real-life contexts. *NeuroImage, 62*(2), 1272-1278. https://doi.org/10.1016/j.neuroimage.2012.02.004

Hayes, A. F. (2018). Partial, conditional, and moderated moderated mediation: Quantification, inference, and interpretation. *Communication Monographs, 85*, 4-40. https://doi.org/10.1080/03637751.2017.1352100

Heller, A. S., Fox, A. S., Wing, E. K., McQuisition, K. M., Vack, N. J., & Davidson, R. J. (2015). The neurodynamics of affect in the laboratory predicts persistence of real-world emotional responses. *Journal of Neuroscience, 35*(29), 10503-10509. https://doi.org/10.1523/jneurosci.0569-15.2015

Hodges, E.V., Boivin, M., Vitaro, F., Bukowski, W.M. (1999). The power of friendship: protection against an escalating cycle of peer victimization. *Dev Psych, 35*(1), 94-101. https://doi.org/10.1037/0012-1649.35.1.94

Jacobs, E., & D'Esposito, M. (2011). Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *Journal of Neuroscience, 31*(14), 5286-5293. https://doi.org/10.1523/jneurosci.6394-10.2011

Jarcho, J.M., Leibenluft, E., Walker, O.L., Fox, N.A., Pine, D.S., Nelson, E.E. (2013). Neuroimaging studies of pediatric social anxiety: paradigms, pitfalls and a new direction for investigating the neural mechanisms. *Biology of Mood & Anxiety Disorders, 3*(1), 14. https://doi.org/10.1186/2045-5380-3-14

Joel, D., Niv, Y., & Ruppin, E. (2002). Actor–critic models of the basal ganglia: New anatomical and computational perspectives. *Neural Networks, 15*(4-6), 535-547. https://doi.org/10.1016/s0893-6080(02)00047-3

Kaltiala-Heino, R., Marttunen, M., Rantanen, P., & Rimpelä, M. (2003). Early puberty is associated with mental health problems in middle adolescence. *Social Science & Medicine, 57*(6), 1055-1064. https://doi.org/10.1016/s0277-9536(02)00480-x

Kashdan, T. B., & Herbert, J. D. (2001). Social anxiety disorder in childhood and adolescence: Current status and future directions. *Clinical Child and Family Psychology Review, 4*(1), 37-61.

Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., Williamson, D., Ryan, N. (1997). Schedule for Affective Disorders and Schizophrenia for School-Age Children-

Present and Lifetime Version (K-SADS-PL): Initial reliability and validity data. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(7), 980-988. https://doi.org/10.1097/00004583-199707000-00021

King-Casas, B., Tomlin, D., Anen, C., Camerer, C. F., Quartz, S. R., & Montague, P. R. (2005). Getting to know you: reputation and trust in a two-person economic exchange. *Science, 308(5718),* 78-83. https://doi.org/10.1126/science.1108062

Kingery, J. N., Erdley, C. A., Marshall, K. C., Whitaker, K. G., Reuter, T. R. (2010). Peer experiences of anxious and socially withdrawn youth: An integrative review of the developmental and clinical literature. *Clinical Child and Family Psychology Review, 13*(1), 91–128. https://doi.org/10.1007/s10567-009-0063-2

La Greca, A. M., Harrison, H. M. (2005). Adolescent peer relations, friendships, and romantic relationships: Do they predict social anxiety and depression? *Journal of Clinical Child and Adolescent Psychology, 34*(1), 49–61. https://doi.org/10.1207/s15374424jccp3401_5

La Greca, A.M., Lopez, N. (1998). Social anxiety among adolescents: Linkages with peer relations and friendships. *J Abnorm Child Psychol, 26*(2): 83-94.

Lau, J.Y.F., Guyer, A.E., Tone, E.B., Jenness, J., Parrish, J.M., Pine, D.S., Nelson, E.E. (2012). Neural responses to peer rejection in anxious adolescents: Contributions from the amygdala-hippocampal complex. *International Journal of Behavioral Development,* 36(1), 36-44. https://doi.org/10.1177/0165025411406854

Masten, C.L., Eisenberger, N.I., Borofsky, L.A., Pfeifer, J.H., McNealy, K., Mazziotta, J.C., Dapretto, M. (2009). Neural correlates of social exclusion during adolescence: understanding the distress of peer rejection. *Social Cognitive and Affective Neuroscience, 4*(2), 143-157. https://doi.org/10.1093/scan/nsp007

Masten, C. L., Telzer, E. H., Fuligni, A. J., Lieberman, M. D., & Eisenberger, N. I. (2012). Time spent with friends in adolescence relates to less neural sensitivity to later peer rejection. *Social Cognitive and Affective Neuroscience*, *7*(1), 106-114. https://doi.org/10.1093/scan/nsq098

McCrimmon, A.W., Smith, A.D. (2013). Review of the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II). *Journal of Psychoeducational Assessment, 31*(3), 337-341. https://doi.org/10.1177/0734282912467756

McDonald, J.H. (2014). *Handbook of biological statistics (3rd ed.).* Baltimore, MD: Sparky House Publishing.

Mendle, J. (2014). Why puberty matters for psychopathology. *Child Development Perspectives*, *8*(4), 218-222. https://doi.org/10.1111/cdep.12092

Merikangas, K.R., He, J., Burstein, M., Swanson, S.A., Avenevoli, S., Cui, L., Benjet, C., Georgiades, K., Swendsen, J. (2010). Lifetime prevalence of mental disorders in U.S. adolescents: Results from the National Comorbidity Study- Adolescent Supplement (NCS-

A). *J Am Acad Child Adolesc Psychiatry, 49*(10), 980-989. https://doi.org/10.1016/j.jaac.2010.05.017

Muthén, L.K., Muthén, B.O. (2015). Mplus User's Guide, Version 8.

Monga, S., Birmaher, B., Chiappetta, L., Brent, D., Kaufman, J., Cully, M. (2000). Screen for Child Anxiety-Related Emotional Disorders (SCARED): Convergent and divergent validity. *Depression and Anxiety*, *12*(2), 85-91. https://doi.org/10.1002/1520-6394(2000)12:2%3C85::aid-da4%3E3.0.co;2-2

Muris, P., & Meesters, C. (2009). Reactive and regulative temperament in youths: psychometric evaluation of the Early Adolescent Temperament Questionnaire-Revised. *Journal of Psychopathology and Behavioral Assessment, 31*(1), 7-19. https://doi.org/10.1007/s10862-008-9089-x

Nelson, E. E., Leibenluft, E., McClure, E. B., & Pine, D. S. (2005). The social re-orientation of adolescence: a neuroscience perspective on the process and its relation to psychopathology. *Psychological Medicine, 35*(2), 163-174. https://doi.org/10.1017/s0033291704003915

O'Doherty, J., Dayan, P., Schultz, J., Deichmann, R., Friston, K., & Dolan, R. J. (2004). Dissociable roles of ventral and dorsal striatum in instrumental conditioning. *Science, 304*(5669), 452-454. https://doi.org/10.1126/science.1094285

Peters, E., Risken-Walraven, J.M., Cillessen, A.H.N, de Weerth, C. (2011). Peer rejection and HPA activity in middle childhood: friendship makes a difference. *Child Development, 82*(6), 1906-1920. https://doi.org/10.1111/j.1467-8624.2011.01647.x

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence, 17*(2), 117-133. https://doi.org/10.1007/bf01537962

Platt, B., Kadosh, K.C., Lau, J.Y.F. (2013). The role of peer rejection in adolescent depression. *Depression and Anxiety, 30*, 809-821. https://doi.org/10.1002/da.22120

Price, R. B., Allen, K. B., Silk, J. S., Ladouceur, C. D., Ryan, N. D., Dahl, R. E., ... & Siegle, G. J. (2016). Vigilance in the laboratory predicts avoidance in the real world: A dimensional analysis of neural, behavioral, and ecological momentary data in anxious youth. *Developmental Cognitive Neuroscience, 19*, 128-136. https://doi.org/10.1016/j.dcn.2016.03.001

Rogol, A. D., Roemmich, J. N., & Clark, P. A. (2002). Growth at puberty. *Journal of Adolescent Health, 31*(6), 192-200. https://doi.org/10.1016/s1054-139x(02)00485-8

Romeo, R. D., & Sisk, C. L. (2001). Pubertal and seasonal plasticity in the amygdala. *Brain Research, 889*(1), 71-77. https://doi.org/10.1016/s0006-8993(00)03111-5

Rucker, D. D., Preacher, K. J., Tormala, Z. L., & Petty, R. E. (2011). Mediation analysis in social psychology: Current practices and new recommendations. *Social and Personality Psychology Compass, 5*(6), 359-371. https://doi.org/10.1111/j.1751-9004.2011.00355.x

Rudolph, K.D., Conley, C.S. (2005). The socioemotional costs and benefits of social-evaluative concerns: Do girls care too much? *J Pers, 73*(1), 115-138. https://doi.org/10.1111/j.1467-6494.2004.00306.x

Rudolph, K.D., Miernicki, M.E., Troop-Gordon, W., Davis, M.M., Telzer, E.H. (2016). Adding insult to injury: neural sensitivity to social exclusion is associated with internalizing symptoms in chronically peer-victimized girls. *Social Cognitive and Affective Neuroscience,* 829-842. https://doi.org/10.1093/scan/nsw021

Sebastian, C. L., Tan, G. C., Roiser, J. P., Viding, E., Dumontheil, I., & Blakemore, S. J. (2011). Developmental influences on the neural bases of responses to social rejection: implications of social neuroscience for education. *Neuroimage*, *57*(3), 686-694. https://doi.org/10.1016/j.neuroimage.2010.09.063

Shiffman, S., Stone, A.A., Hufford, M.R. (2007). Ecological momentary assessment. *Annual Review of Clinical Psychology, 4,* 1-32. https://doi.org/10.1146/annurev.clinpsy.3.022806.091415

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: correspondence between hormonal and physical development. *Child Development*, *80*(2), 327-337. https://doi.org/10.1111/j.1467-8624.2009.01263.x

Siegel, R. S., La Greca, A. M., & Harrison, H. M. (2009). Peer victimization and social anxiety in adolescents: Prospective and reciprocal relationships. *Journal of Youth and Adolescence, 38*(8), 1096-1109. https://doi.org/10.1007/s10964-009-9392-1

Silk, J.S., Siegle, G.J, Lee, K.H., Nelson, E.E., Stroud, L.R., Dahl, R.E. (2014). Increased neural response to peer rejection associated with adolescent depression and pubertal development. *Social Cognitive and Affective Neuroscience, 9,* 1798-1807. https://doi.org/10.1093/scan/nst175

Silk, J.S., Stroud, L.R., Siegle, G.J., Dahl, R.E., Lee, K.H., Nelson, E.E. (2012). Peer acceptance and rejection through the eyes of youth: pupillary, eyetracking and ecological data from the Chatroom Interact task. *Social Cognitive and Affective Neuroscience, 7,* 93-105. https://doi.org/10.1093/scan/nsr044

Slavich, G. M., Way, B. M., Eisenberger, N. I., & Taylor, S. E. (2010). Neural sensitivity to social rejection is associated with inflammatory responses to social stress. *Proceedings of the National Academy of Sciences, 107(33), 14817-14822*. https://doi.org/10.1073/pnas.1009164107

Steinberg, L., Morris, A.S. (2001). Adolescent development. *Annual Reviews of Psychology, 52,* 83-110. https://doi.org/10.1146/annurev.psych.52.1.83

Swartz, J. R., Prather, A. A., Di Iorio, C. R., Bogdan, R., & Hariri, A. R. (2017). A functional interleukin-18 haplotype predicts depression and anxiety through increased threat-related amygdala reactivity in women but not men. *Neuropsychopharmacology, 42*(2), 419-426. https://doi.org/10.1038/npp.2016.129

Tricomi, E., Delgado, M. R., McCandliss, B. D., McClelland, J. L., & Fiez, J. A. (2006). Performance feedback drives caudate activation in a phonological learning task. *Journal of Cognitive Neuroscience, 18*(6), 1029-1043. https://doi.org/10.1162/jocn.2006.18.6.1029

Vaden Jr, K. I., Gebregziabher, M., Kuchinsky, S. E., & Eckert, M. A. (2012). Multiple imputation of missing fMRI data in whole brain analysis. *Neuroimage, 60*(3), 1843-1855. https://doi.org/10.1016/j.neuroimage.2012.01.123

Velting, O. N., & Albano, A. M. (2001). Current trends in the understanding and treatment of social phobia in youth. *The Journal of Child Psychology and Psychiatry and Allied Disciplines*, *42*(1), 127-140. https://doi.org/10.1111/1469-7610.00705

Wechsler, D. (2011). *Wechsler Abbreviated Scale of Intelligence–Second Edition (WASI-II).* San Antonio, TX: NCS Pearson. https://doi.org/10.1037/t15171-000

Will, G-J., van Lier, P., Crone, E.A., Guroglu, B. (2016). Chronic childhood peer rejection is associated with heightened neural response to social exclusion during adolescence. *J Abnorm Child Psychol, 44*, 43-55. https://doi.org/10.1007/s10802-015-9983-0

Wilson, S. J., Smyth, J. M., & MacLean, R. R. (2014). Integrating ecological momentary assessment and functional brain imaging methods: new avenues for studying and treating tobacco dependence. *Nicotine & Tobacco Research*, *16*(Suppl_2), S102-S110. https://doi.org/10.1093/ntr/ntt129

Zimbardo, P. G., & Radl, S. (1981). *The shy child*. New York: McGraw-Hill.