Testing a Neurobiological Susceptibility to Social Context Model Linking Neural Reward Processing and Social Stress to Social Anxiety in Adolescent Girls

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Social anxiety disorder (SAD) is one of the most common and impairing disorders in adolescence, particularly for girls, and remains one of the most challenging disorders to treat. A better understanding of the mechanisms supporting the development of SAD in adolescence is important for identifying new targets for intervention. Emerging research and theory rooted in a neurobiological susceptibility to social context framework suggest that interactions between neural reward function and adverse social environments are key for understanding the etiology of SAD. Backed by this research and theory, this project employed ecologically-valid methods at multiple levels of analysis to examine how perceptions of socially threatening interactions with peers in daily life (assessed using ecological momentary assessment) interact with neural reward function to confer risk for social anxiety symptoms in 129 girls (ages 11-13) at temperamental risk for SAD.

In support of the primary hypothesis, activation in the basolateral amygdala (BLA) to the anticipation of socially rewarding (vs. neutral) feedback interacted with daily social threat at baseline to predict social anxiety symptom severity two years later. A positive association between social threat and social anxiety symptoms was only seen for girls with high BLA activity. Findings were specific to the BLA (vs. a more distributed social reward network) and to neural activation to social reward (vs. threat) anticipation. Unexpectedly, interactions between daily social threat and BLA activation to social reward anticipation at baseline also predicted symptoms of generalized anxiety and depression two years later, suggesting that these processes may serve as
transdiagnostic risk factors for internalizing disorders. Findings suggest that socially threatening experiences are particularly detrimental during adolescence for youth highly sensitive to reward contingencies, potentially due to effects on reward learning processes. More generally, results add to a growing literature highlighting the importance of neural reward function in the development of social anxiety and other internalizing disorders during adolescence.
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Preface

I would like to acknowledge my fantastic mentors, colleagues, mentees, family members, and friends who have supported me throughout my academic journey. I would further like to thank my mentor, Dr. Jennifer Silk, for her endless support and advice. Jen exudes generosity, humility, and kindness. She is an incredible role model in every way, and I leave each interaction with her feeling confident, capable, passionate, heard, and respected. I am also so fortunate to have been part of the FEND Lab, and am grateful for all the amazing research assistants and undergraduate students who are so dedicated and hard-working, and bring such joy and positive energy to lab. Additionally, I would like to thank the participants and their families for contributing to this work.

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1.0 Introduction

Julie, a 14-year-old female, agreed to start therapy because she is tired of feeling uncomfortable around her peers and skipping fun events, such as school dances and birthday parties, because she is worried about other teens judging her. Julie reports that for several years she has wanted to make new friends, join the photography club, and get back into soccer, but she has “always” been too shy and “knows” that she will humiliate herself if she tries to interact with others. She is hesitant to set therapy goals for socializing and frequently cites bullying from her peers a few years ago as reasons against setting these goals.

In line with classic theories of fear and anxiety (for a review see Mogg & Bradley, 1998), Julie may be displaying hypersensitivity to threat in her environment, including cognitive and attentional biases toward threat. Heightened threat sensitivity could be supported by dysfunction in her brain’s threat circuitry (e.g., amygdala, prefrontal cortex) (Davidson, 2002). In cognitive behavioral therapy (CBT), the therapist might help Julie identify and challenge her threat-related biases. Exposures are also likely to be used to reduce the fear response through inhibitory learning processes.

Julie’s threat reactivity is important for understanding her social anxiety, but is it enough? Recent research and theory suggests that reward function may also be important for understanding social anxiety in adolescence (e.g., Bar-Haim et al., 2009; Richey et al., 2019). Moreover, heightened neural responses to reward anticipation in children and adolescents at temperamental risk for social anxiety (e.g., shy or fearful) may be one specific factor that contributes to the development of social anxiety symptoms in later adolescence. Yet we still know little about how and why neural reward functioning confers risk for social anxiety during adolescence. Further, it
is unlikely that neural reward function alone confers risk for social anxiety; as with most developmental processes, it is important to consider both biology and the environment. For social anxiety, the social environment may be particularly relevant to consider. Many shy youth, like Julie, report more negative interactions with their peers. However, some shy youth form and maintain close friendships and experience little victimization from peers. Aligning with recent neurobiological susceptibility to social context models (Richey et al., 2019; Schriber & Guyer, 2016), one possibility is that social anxiety emerges during adolescence from interactions between high sensitivity to potential reward and high social stress, potentially through effects of social stress on reward learning mechanisms. This could help explain why not all youth who are temperamentally shy or behaviorally inhibited in early life develop social anxiety later in life (Sandstrom et al., 2020).

Testing this neurobiological susceptibility to social context theory empirically is a critical next step to advance the field and move closer to understanding the role of reward function in social anxiety development. To best test this theory, we need large samples, longitudinal data, and ecologically valid methods. The present dissertation addresses these needs by examining how heightened neural reactivity to the anticipation of social rewards (measured using a novel peer interactive task) interacts with perceptions of social threat in daily life to confer risk for social anxiety symptoms over a two-year period in adolescence. The sample was restricted to adolescent girls oversampled for shy or fearful temperament, as this population is at heightened risk for SAD (Sandstrom et al., 2020). One important question that this dissertation also addresses is specificity to reward anticipation. Some research suggests that youth at temperamental risk for social anxiety show heightened neural responses not only to potential rewards but also to potential punishments (Guyer et al., 2006, 2012), which could suggest that heightened neural activity to the anticipation
of incentives is not about the reward *per se*, but more about anticipating uncertain outcomes or attending to performance-related contingencies. The present research is critical for understanding whether and how reward function is really implicated in youth social anxiety development.

### 1.1 Adolescent Social Anxiety Development

Understanding how and why social anxiety increases during adolescence has serious implications for youths’ mental and physical health. Anxiety disorders are among the most common and impairing psychiatric disorders in childhood and adolescence. Social anxiety in adolescence predicts academic underachievement, smaller social networks, and poorer social skills (Ginsburg et al., 1998; Kashdan & Herbert, 2001). Youth with social anxiety also often experience high levels of loneliness, dysphoria, and generalized anxiety (Beidel, Turner, & Morris, 1999), as well as comorbid depressive disorders and substance use disorders (Essau, Conradt, & Petermann, 1999).

Rates of social anxiety disorder (SAD) skyrocket in early adolescence, particularly for girls, with the highest rates of SAD seen between the ages of 10 and 19 years (Beesdo et al., 2007). By age 16, almost one in 10 teens will have met diagnostic criteria for SAD (Merikangas et al., 2010). Many children and adolescents also experience distressing and functionally impairing symptoms of social anxiety but do not meet full diagnostic criteria for SAD; girls may be at particularly high risk for increases in subclinical social anxiety symptoms around ages 14 to 15 years (Ranta et al., 2007).

One of the best predictors of social anxiety disorder in adolescence is early life temperament. Behavioral inhibition (BI) is a biologically-based temperament that can be
characterized as early as infancy by a fear of novelty (Kagan et al., 1984). School-age children high in BI typically appear shy, fearful, and socially reticent. Research suggests that high BI in childhood is associated with almost a six-fold increase in odds of SAD in adolescence (Sandstrom et al., 2020). However, the mechanisms through which shy, fearful, inhibited temperament confer risk for SAD are still being uncovered. Thus, reliable early interventions have yet to be established but are sorely needed due to low rates of SAD treatment response (Walkup et al., 2008). As discussed in the following sections, recent research suggests that reward responsiveness, which develops around the same time that increases in social anxiety are occurring, may be one key factor for understanding why youth high in shy or fearful temperament are at greater risk for SAD.

1.2 Reward Responsiveness

Reward responsiveness is one construct encompassed in the positive valence system (PVS) functional domain defined by the National Institute of Health’s Research Domain Criteria (RDoC). The present dissertation focuses on reward anticipation, a subconstruct of reward responsiveness. Behaviorally, reward anticipation refers to the ability to “anticipate and/or represent a future incentive” (RDoC, NIMH). Reward anticipation can be distinguished from other reward states (i.e., consummation and learning) based on neurobiology, as discussed in foundational research by Berridge and colleagues (Berridge, 2003). According to Berridge and colleagues (Berridge, 2003; Berridge, Robinson, & Aldridge, 2009), dopamine is commonly linked to the approach motivational system and reward anticipation or “wanting”. The neural substrates of reward “wanting” are widely distributed (Berridge et al., 2009) and include mesocorticolimbic dopaminergic projections from the midbrain (e.g., ventral tegmental area, VTA) to the nucleus
accumbens (NAcc), amygdala, and regions of the prefrontal cortex (Berridge & Robinson, 2016). Indeed, meta-analyses of neural reward processing in adolescents and adults have shown that the NAcc and other regions of the striatum (i.e., caudate and putamen), as well as the amygdala, are engaged during monetary reward anticipation (Oldham et al., 2018; Silverman et al., 2015).

The thalamus and anterior insula (AI) are also reliably engaged when adolescents and adults anticipate monetary rewards (Cao et al., 2019; Oldham et al., 2018; Silverman et al., 2015). Neurons in the thalamus receive input from the VTA/substantia nigra and send projections to the striatum and/or prefrontal cortex. Neurons in the mediodorsal nucleus of the thalamus specifically have been shown to respond strongly to cues associated with a reward, supporting stimulus-reward learning mechanisms (e.g., Kawagoe et al., 2007; Yu, Gupta, & Yin, 2010). The AI is involved in salience detection and may play a role in integrating interoceptive information to judge reward values, thus playing a role in reward decision-making (Wittmann et al., 2010). The striatum (i.e., NAcc, caudate, and putamen), thalamus, amygdala, and AI are also reliably activated when an individual anticipates a potential monetary loss (Oldham et al., 2018).

The striatum, amygdala, thalamus, and AI are also engaged when individuals anticipate social rewards specifically (Kohls et al., 2013; Martins et al., 2021; Rademacher et al., 2010, 2014; Spreckelmeyer et al., 2009). Other regions involved in social reward anticipation include the precuneus and dorsal anterior cingulate cortex (dACC; Gu et al., 2019; Martins et al., 2021; Rademacher et al., 2010; Sprecklemeyer et al., 2009). The precuneus is a major hub of the default-mode network and plays a key role in social-cognitive processes (Uddin et al., 2007), including self-referential processing and social valuation (Kumar et al., 2019). The dACC, like the AI, is an important region of the salience network (Seeley, 2019). Whereas the insula is critical for perceiving visceral feedback, the dACC is critical for generating autonomic, behavioral, and
cognitive responses (Seeley, 2019). These regions interact to form an “information processing loop” responsible for responding to salient internal and external stimuli and assigning these stimuli emotional weight (Seeley, 2019). Interestingly, though the precuneus and dACC are not typically considered core regions of the reward brain network, in a sample of over 1500 adolescents around age 14, Cao et al. (2018) found that the precuneus and dACC were significantly engaged during monetary reward anticipation, potentially related to their roles in the default mode network and salience network, respectively.

1.2.1 Reward Responsiveness: Typical Adolescent Development

Neural reward responsiveness may be an important factor to consider in relation to adolescent social anxiety given the substantial changes that occur in the brain’s reward circuitry during this sensitive period of development (see reviews by Forbes & Goodman, 2014; Gilbert, 2012). For example, a significant increase in dopaminergic projections to the medial PFC and changes in dopaminergic receptor expression are seen during adolescence (see Walker et al., 2017). Additionally, in several widely-cited and influential “dual systems” models, adolescence has been characterized by earlier development of brain regions implicated in approach motivation and reward seeking (e.g., NAcc) and later development of regions involved in emotion regulation and cognitive control (e.g., dorsomedial PFC) (Casey, Getz, & Galván, 2008; Ernst et al., 2009). Measures of functional connectivity suggest that relatively stable coupling between the PFC and subcortical structures, including the VS, do not occur until the mid-20s (Dosenbach et al., 2010). Though many of these neural changes have been linked specifically to changes in chronological age, puberty likely does play a role in the development of the neural reward system. Puberty refers to the physiological and behavioral changes associated with reproductive competence, such as
activation of the hypothalamic-pituitary-gonadal axis and increases in sex steroid hormones (Crone & Dahl, 2012; Walker et al., 2017). Sex steroid hormones may affect behavior at puberty through specific brain structures associated with socio-affective learning, including the amygdala (Romeo & Sisk, 2001).

These reward-related neurobiological changes are believed to underlie increases in reward sensitivity and support heightened approach motivation and reward-seeking behaviors during adolescence (Ernst, Romeo, & Anderson, 2009; Paus, 2005; Steinberg, 2008; van Duijvenvoorde et al., 2016). Research shows that adolescents prefer short-term over delayed rewards (Steinberg et al., 2009), are more likely to seek rewards in the context of high reward potential relative to children and adults (Cauffman et al., 2010), and are faster to approach emotional information than children and adults (Tottenham et al., 2011). Most research supports the theory that adolescent reward behavior is driven by hyper-responsiveness of the striatal reward system, which may be specific to the anticipation of reward (Galván, 2010; Geier et al., 2010). Heightened reward seeking in adolescence may lay the groundwork for adolescents to try new things and forge new relationships, critical tasks for this developmental stage.

1.2.2 Studying Reward Responsiveness in Adolescence

Reward anticipation (and reward responsiveness more broadly) has been studied using a variety of methods in adolescent samples. Questionnaire measures such as Carver and White’s (1994) Behavioral Inhibition System/Behavioral Activation System (BIS/BAS) scale can be used to measure adolescent self-reported or parent-reported approach motivation. Behavioral tasks can also be used to measure how the presence of potential rewards influences performance on a task or decision-making. For example, Jazbec et al. (2005) used a cognitive control task to examine
how the presentation of incentives prior to the task influences behavioral performance in youth with anxiety disorders. Behavioral reward tasks can also be administered while measuring brain activity to examine neural correlates of reward responsiveness; in youth, this is often done using functional magnetic resonance imaging (fMRI) or electroencephalography (EEG).

Many fMRI and EEG studies on reward anticipation in adolescent samples measure neural activity while youth anticipate or receive monetary rewards. For example, the monetary incentive delay (MID) task (Knutson et al., 2000) is commonly used to study neural activation to the anticipation of monetary rewards. In this task, participants view a shape at the start of each trial that cues them of the potential outcomes they can receive if they respond to a target that flashes on the screen in fast enough time. One shape cues the participant that if they respond fast enough, they will gain money, while if they are too slow, they will neither gain nor lose money (i.e., the reward anticipation cue). Other shapes cue the participant of potential monetary loss if they are too slow (i.e., the punishment anticipation cue) or cue the participant that they will neither gain nor lose money on this trial (i.e., neutral cue).

Given that adolescence is a period of heightened social processing (Blakemore & Mills, 2014), monetary rewards may not be as salient or motivating for adolescents as social rewards. To assess social reward processing, a social adaptation of the MID task – the Social Incentive Delay (SID) task – was developed (Spreckelmeyer et al., 2009). The behavioral component of the task is the same, but the rewards offered based on the participant’s performance are social (i.e., happy, angry, or neutral faces) rather than monetary (i.e., win, loss, no change). Studies have found that similar neural regions respond to the anticipation of monetary and social rewards on the MID and SID tasks, including the ventral striatum, anterior insula, thalamus, and amygdala (Martins et al., 2021; Rademacher et al., 2010).
To my knowledge, only one study has used the SID task to study neural reward responsivity in youth with (or at risk for) social anxiety (Kaurin et al., 2022). Neural reactivity to the anticipation of other forms of socially rewarding stimuli (e.g., positive social feedback) in adolescents with anxiety disorders or symptoms, or at temperamental risk for social anxiety, has instead been studied using a variety of socially interactive tasks, including the Cyberball task (Williams & Jarvis, 2006), the original Chatroom task (Guyer et al., 2008), the Chatroom Interact task (Silk et al., 2012b), and the Virtual School task (Jarcho et al., 2015). These tasks measure neural responses to the anticipation or receipt of social inclusion or positive social evaluation/feedback (e.g., acceptance, compliments). While ecologically valid, these socially interactive tasks vary conceptually from the MID task. For example, an adolescent who is anticipating feedback on the Chatroom task may think about how their appearance or personal characteristics might elicit this feedback, whereas anticipating monetary rewards on the MID task is directly tied to behavioral performance. Thus, it is difficult, if not impossible, to compare findings using the MID task to findings using the Cyberball, Chatroom, or Virtual School tasks (see Sequeira et al., 2022). Using social reward tasks that more closely mimic common monetary reward tasks (like the SID task) can aid in our ability to more directly compare social vs. non-social reward processing in adolescence.

1.2.3 Reward Responsiveness: Links to Adolescent Social Anxiety

The majority of research support for altered reward function as a factor contributing to the development of social anxiety during adolescence comes from research using fMRI to study neural activity during the anticipation or receipt of social and non-social rewards in children high in BI temperament, a risk factor for future SAD (Sandstrom et al., 2020). High BI in childhood has been
repeatedly associated with increased activity in the striatum to the anticipation of monetary rewards when rewards are contingent on task performance (Bar-Haim et al., 2009; Guyer et al., 2006). Of note, similar patterns of striatal activity have been found during the anticipation of potential monetary losses (Guyer et al., 2006), which could suggest that high striatal sensitivity to incentives underlies performance-related concerns in youth at risk for SAD. High striatal activation to potential rewards or losses that are contingent on one’s behavior could also signal heightened vigilance toward evaluating the consequences of one’s actions, which could facilitate learning (Caouette & Guyer, 2014). Comparable findings have been shown when late adolescents with a history of BI anticipate social evaluative feedback on the Chatroom Task (Guyer et al., 2014), such that youth with a history of BI show enhanced activity in the dorsal striatum when anticipating social feedback from peers they are most interested in chatting with.

Importantly, one study has also shown that high activity to the anticipation of monetary rewards in the caudate, a region of the striatum, confers risk for future social anxiety symptoms in youth high in BI (Pérez-Edgar et al., 2014). Additionally, youth with social anxiety disorder show heightened sensitivity to the anticipation of incentives that increase in magnitude (Guyer et al., 2012); this pattern of findings was not seen in youth with generalized anxiety disorder. Further, associations between high striatal activity to incentive anticipation and general anxiety symptoms were not found in a larger community sample of youth not recruited on the basis of BI (Mikita et al., 2016). Taken together, findings suggest that high striatal activation to reward anticipation is a mechanism more specific to the development of SAD, and one that forms during childhood in youth at temperamental risk for social anxiety.

These findings show promise for studying reward responsiveness as a factor that contributes to SAD development. However, several limitations of the literature linking neural
reactivity to reward anticipation to BI or social anxiety exist and are important to address. First, only two studies have actually linked high neural activation to the anticipation of social or non-social rewards to increases in social anxiety symptoms over time. Second, most existing studies on neural activation to reward anticipation in BI youth have used the same sample of youth recruited from the mid-Atlantic United States, calling into question the generalizability of these findings. Moreover, it remains unclear whether high neural reactivity to reward anticipation may support the development of social anxiety in temperamentally shy or fearful children not identified as high BI from infancy (or whether this mechanism is specific to BI). Finally, most existing research on neural activation to reward anticipation in BI youth has been done using a version of the MID task. Though research has also found heightened neural activity when youth with a history of BI or social reticence (SR) anticipate uncertain or highly valued feedback from peers on the Chatroom task or Virtual School (e.g., Clarkson et al., 2019; Guyer et al., 2014; Jarcho et al., 2015), these social tasks differ substantially from the MID task, making it difficult to compare findings.

1.3 Adolescent Social Development

Coinciding with the development of neural reward circuitry during adolescence are significant changes in the adolescent’s social environment that may work independently or with changes in neural reward function to increase risk for social anxiety. The transition to adolescence is characterized, in part, by a change in dependency on parents to peers (Allen & Antonishak, 2008). Classmates and friends begin to fulfill needs for intimacy, companionship, and reinforcement of personal worth that were previously fulfilled by parents (Rubin, Bukowsky, & Parker, 2006). Peers play a central role in socialization of the adolescent by helping the adolescent
understand their social world (Piaget, 1950), develop a coherent identity (Erikson, 1968), and prepare for romantic relationships (Sullivan, 1953). Co-occurring with increased dependence on peers is, unsurprisingly, increased time spent with peers. By middle childhood, peer interactions make up over 30% of a child’s social interactions (Gifford-Smith & Brownell, 2003). For adolescents with access to cell phones and/or internet, this percentage is likely much higher. With the proliferation of texting and social media platforms, peers can remain virtually connected at almost all times throughout the day. More time spent with peers could bring more opportunity for conflict with peers, and around 10-15% of adolescents experience stable levels of bullying and victimization (Troop-Gordon, 2017).

Another aspect of the social shift occurring during adolescence is increased social awareness, or awareness of how one’s traits are viewed by others, which contributes to increased concern of the opinions of others (Harter, 2016). Heightened attention to peer opinions and peer acceptance is likely related to increases in neural sensitivity to social evaluation occurring during this developmental period (Nelson, Jarcho, & Guyer, 2016). A handful of neuroimaging studies have shown heightened activity in a socio-affective neural network, including the amygdala, dACC, dorsal and ventral striatum, and AI, while adolescents anticipate and receive acceptance and rejection feedback from their peers (e.g., Guyer et al., 2008, 2009, 2014; Jarcho et al., 2015, 2016). Heightened neural activation to social evaluation is likely related to the normative maturation in cortico-subcortical neural circuitry occurring during adolescence, briefly reviewed in Section 1.2.1.
1.3.1 Social Development: Links to Adolescent Social Anxiety

Youth high in shyness or social anxiety often report more negative, and less positive, peer relationships. Shy adolescents may be particularly sensitive to social interactions and may also have more trouble interacting or connecting with peers, contributing to lower quality friendships and more peer rejection (Degnan, Almas, & Fox, 2010). Children who perceive themselves as more socially accepted also report lower levels of social anxiety (Festa & Ginsburg, 2011), while highly socially anxious adolescents report lower levels of social acceptance and more negative peer interactions (Erath, Flanagan, & Bierman, 2007; Ginsburg, La Greca, & Silverman, 1998). Notably, peer stress, but not family stress, has been shown to predict increases in social anxiety symptoms over time during adolescence (e.g., Epkins & Heckler, 2011; Griffith et al., 2020).

1.3.2 Studying Social Relationships in Adolescence

One limitation of the literature linking social stress to social anxiety is the predominance of one-time questionnaire measures that rely on adolescents remembering their peer relationships from weeks, months, or even years prior. This is clearly subject to retrospective biases, and may be influenced by the adolescent’s current functioning (e.g., adolescents who are more socially anxious may report that their past interactions were worse than they felt in the moment). This highlights a need for more ecologically valid measures of adolescents’ peer relationships that do not rely so heavily on retrospective reporting.

Ecological momentary assessment (EMA) is a promising approach to studying adolescents’ perceptions of their social relationships in a way that reduces retrospective bias. Using EMA allows one to study behavioral, affective, and situational variables with rich insight into
naturalistic conditions (Wilson et al., 2014). EMA also allows for a more stable measure of functioning, as the same variables can be collected several times over a period of days, weeks, or months. For example, an adolescent girl reporting a strong negative reaction to a peer interaction on the first day and more mild responses the next two weeks is likely functioning differently socially than a girl reporting strong negative reactions to peer interactions every day. If perceptions of peer interactions were only collected on the first day, these girls might look very similar. On the other hand, using EMA to study their perceptions over a two-week period is likely to provide a more accurate picture of their social functioning.

1.4 Neural Reward Responsiveness and Peer Stress: Relevant Theoretical Models for Social Anxiety Development

As youth high in shy or inhibited temperament show heightened engagement in socio-affective regions during reward anticipation, report more peer rejection, and are at heightened risk for social anxiety, one hypothesis could be that heightened reward sensitivity interacts with more negative peer experiences in shy youth to confer risk for social anxiety. This hypothesis is echoed in several recent developmental theories of social anxiety disorder (e.g., Barker, Buzzell, & Fox, 2019; Caouette & Guyer, 2014; Richey et al., 2019; Silk et al., 2012a). For example, according to approach-avoidance conflict models (e.g., Barker et al., 2019; Caouette & Guyer, 2014; Helfinstein, Fox, & Pine, 2012), heightened activity in both the behavioral inhibition system (BIS) and behavioral activation system (BAS), and conflict between these systems, characterizes high BI. This model is rooted in reinforcement sensitivity theory (Gray, 1987), which proposes two orthogonal motivational systems that explain behavior: the BIS and BAS. Individuals with high
BAS reactivity are more prone to engage in approach behavior while people high in BIS reactivity tend towards cautiousness and avoidance behavior (Carver & White, 1994). For example, a fearful child entering a novel social environment, such as a school dance, may feel highly motivated to seek out positive social experiences with peers. However, a co-occurring fear of embarrassment and rejection fuels the avoidance motivational system.

A related model of youth anxiety proposed by Silk and colleagues (2012a) considers how heightened threat sensitivity in children with, or at risk for, anxiety disrupts reward seeking in adolescence. The authors suggest that social and neurobiological changes in adolescence may exacerbate conflict between high threat and reward sensitivity in youth with anxiety. Over time, reward seeking and processing in a subset of adolescents with anxiety may become blunted in socially threatening contexts, such as parties and school dances, where the possibility of negative evaluation is high.

These theories consider how neural reactivity to reward anticipation alone is unlikely to predict social anxiety symptoms in temperamentally at-risk youth, which could help explain why not all youth high in BI or social reticence go on to develop social anxiety disorder (Sandstrom et al., 2020). A more likely explanation is that interactions between neural activity and environmental factors (e.g., peer stress) confer risk for social anxiety over time. This is summarized and detailed by the neurobiological susceptibility to social context hypothesis (Schriber & Guyer, 2016). Drawing from the differential susceptibility literature (e.g., Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007), Schriber and Guyer (2016) propose that an adolescent’s level of neurobiological sensitivity moderates the impact of positive or negative social contexts on development. This means that adolescents high in neurobiological sensitivity are most negatively affected by adverse social environments and also most likely to thrive in positive social
environments. Adolescents high in neurobiological sensitivity may thus be at highest risk for psychopathology when they perceive more negative social relationships and at lowest risk for psychopathology when they perceive more positive social relationships.

Informed by the neurobiological susceptibility to social context hypothesis, Richey et al. (2019) proposed the Sensitivity Shift Theory (SST). SST is built on the premise that adolescents high in BI temperament (a risk factor for social anxiety disorder) are both hyper-responsive to their environments (i.e., high neurobiological sensitivity) and disproportionately experience adverse social environments. Richey and colleagues suggest that it is the combination of high neurobiologically-supported responsivity to the environment and negative experiences with peers for early adolescents high in BI that confers risk for social anxiety in mid-adolescence. Moreover, they propose that the link between high neurobiological susceptibility, negative experiences with peers, and social anxiety is supported by altered reinforcement learning mechanisms. Specifically, repeated negative experiences with peers would be expected to modify the strength of the association between environmental social cues and their outcomes, increasing the salience of distressing cues and supporting a conditioned avoidance response to these cues. Adolescence is a sensitive period in which interactions between neural reward circuitry and social stress are likely to have a strong impact, as adolescence is associated with heightened sensitivity to social cues, increases in potentially rewarding social opportunities and peer conflict, and changes in reward-related brain structures and networks that support learning.

For Julie (from the introduction) who reports a history of shyness, high sensitivity to potential rewards in her social environment (e.g., being asked to dance at a school dance, scoring a goal in soccer) in late childhood and early adolescence could enhance her attention to action-outcome contingencies and facilitate learning (Guyer et al., 2012; Richey et al., 2019). In the
presence of peer rejection, however, heightened sensitivity to the anticipation of potential rewards may be detrimental, as Julie may come to associate potentially rewarding situations with threat or failure. Over time, a conditioned avoidance response to social cues that could signal potential reward may develop (e.g., running away from a friend approaching after class), as well as cognitive biases (e.g., seeing the worst in a generally positive social situation). These avoidance responses and cognitive biases could then contribute to more severe symptoms of social anxiety.

1.5 Interactions between Neural Reward Responsiveness and Peer Stress: Limited Empirical Support

These neurobiological susceptibility to social context models also propose that recurrent social stress contributes to social anxiety, in part, because social stress “gets inside the brain,” sensitizing biological systems and shaping neural function (Rudolph et al., 2021). This may be particularly true during adolescence, as the developing adolescent brain is highly sensitive to social stress (Eiland & Romeo, 2013). Indeed, aberrant functioning in brain regions involved in processing social rejection and emotion regulation has been found in youth with a history of peer victimization (McIver et al., 2018; Rudolph et al., 2021; Will et al., 2016). Moreover, Rudolph et al. (2016) found that, in adolescent girls, the association between neural responses to peer exclusion (in the sgACC, dACC, and anterior insula) and symptoms of depression and anxiety was moderated by a history of peer victimization, such that the association between brain function and symptomatology was stronger in chronically victimized than non-victimized girls.

We also know that social stress “gets inside the brain” to impact reward function specifically, which could influence the development of social anxiety. A breadth of human and
non-human animal research has shown that chronic social stress and changes to the social environment (e.g., decreases in playful interactions between adolescent rats) contribute to morphological changes in dopaminergic brain structures (Bell et al., 2010; Ironside et al., 2018) and reorganization of fronto-striatal brain circuitry (Dias-Ferreira et al., 2009). Increases in NAcc dopamine release immediately following social defeat have also been found in adult rats (Tidey & Miczek, 1996). However, repeated social stress could actually lead to decreased dopamine activity in the NAcc (Cabib & Puglisi-Allegra, 2012; Miczek et al., 2011); behaviorally, this could reflect a shift from active coping to learned helplessness (Ironside et al., 2018), potentially setting the stage for more severe social anxiety symptoms.

Social stress may also influence social anxiety through increases in brain-derived neurotrophic factor (BDNF) in the NAcc. Social avoidance is a common behavioral response to social stress that may confer risk for social anxiety. Research in adult rats suggests that increases in BDNF in the NAcc (from the VTA) may mediate the link between social stress and social avoidance, potentially through effects on dopamine neurons (Koo et al., 2016). NAcc BDNF levels may also be influenced by glucocorticoids released by the hypothalamic-pituitary-adrenal (HPA) axis in response to stress (Richey et al., 2019). Given the role of BDNF in synaptic growth and neural plasticity (Binder & Scharfman, 2004), BDNF may support increased neuroplasticity in the NAcc following social stress. Richey et al. (2019) propose that NAcc neuroplasticity could support heightened reinforcement learning under conditions of social stress, contributing to increases in social anxiety symptoms during adolescence. Together, these studies suggest that the interplay between social stress and neural reward circuitry is key for understanding the development of social anxiety.
To my knowledge, though, only one empirical study has actually tested interactions between social stress and neural reward function as a predictor of social anxiety symptoms. Jarcho et al. (2019) examined how neural activation to the anticipation of social rewards in early adolescence interacts with peer victimization to confer risk for social anxiety symptoms in youth at temperamental risk for social anxiety due to early childhood wariness. Using the Virtual School task, Jarcho and colleagues (2019) found that for early adolescents (11 years) experiencing high peer victimization (based on a four-item self-report questionnaire), higher early childhood wariness (at ages 2-7 years) was associated with greater reactivity in the right amygdala to the receipt of unpredictably positive peer feedback at age 11. Moreover, in highly victimized participants, higher early childhood wariness and right amygdala responsivity to the receipt of unpredictably positive peer feedback were associated with more severe symptoms of social anxiety at age 11.

Importantly, findings from this latter study (Jarcho et al., 2019) suggest that for youth at temperamental risk for social anxiety, social stress and neural reward function may interact to predict increases in social anxiety symptoms. Interestingly, though, Jarcho et al.’s (2019) findings were specific to the receipt of unpredictably positive peer feedback. This could be related to the nature of the Virtual School task, such that this task may provide greater insight into how the predictability of social feedback influences brain function, rather than tapping core reward processes. This is because participants learn the “reputations” of the virtual peers they are interacting with before completing the Virtual School task in the MRI scanner. Prior to the scan, participants learn whether these peers have been rated by others as nice (“predictably positive”), mean (“predictably negative”), or unpredictable (sometimes nice, sometimes mean). Learning these reputations before the scan may also make the anticipated or received social feedback feel
less personal, and thus less inherently rewarding or threatening. Moreover, the Virtual School task does not provide a performance component, which may be critical for understanding the role of reward anticipation on social anxiety development. Research has shown that heightened neural sensitivity to reward anticipation in youth at temperamental risk for anxiety is only seen when the rewards are contingent on the participant’s performance (Bar-Haim et al., 2009; Benson et al., 2015). How interactions between social stress and neural activation to the anticipation of socially rewarding feedback delivered based on the participant’s performance influence the development of social anxiety is important to explore in future research.

Several additional limitations of this prior study (Jarcho et al., 2019) also bear mentioning as they can be addressed in future research. First, the size of the same was small (N=47) and though wariness was defined in early childhood, the measures of peer victimization, brain function, and social anxiety were all collected simultaneously. Whether brain function and peer victimization influences social anxiety over time remains a key question to explore, particularly if one wishes to target these variables as an intervention. Second, the sample was split into a high peer victimization and low peer victimization group based on a four-item self-report questionnaire. More ecologically valid measures of peer experiences are crucial to integrate into this work. Finally, social anxiety symptoms were self-reported; associations between social anxiety symptoms and peer victimization could be related to shared method variance.

1.6 Current Study

To advance understanding of the processes through which reward responsiveness and peer stress contribute to the development of social anxiety symptoms in adolescence, we need data
collected at multiple levels of analysis and multiple time points to test models rooted in developmental theory. This dissertation addresses these needs with a multimethod, longitudinal design informed by prior research and theory. In 129 girls oversampled for shy or fearful temperament, perceived social threat in negative interactions with peers was assessed using an ecological momentary assessment (EMA) protocol at baseline (Wave 1). This approach provides information on the participant’s perspective of social threat but is less impacted by retrospective bias. Neural responses to the anticipation of social rewards were measured at baseline using a socially interactive version of the SID task. At baseline and at two-year follow-up (Wave 2), social anxiety symptoms were assessed by clinical interviewers to reduce shared method variance.

The first aim of this study (Aim 1) was to use these ecologically-valid measures to test how the interaction between neural activation to the anticipation of social rewards and perceptions of social threat in early adolescent girls (ages 11-13 years) confers risk for social anxiety symptoms in mid-adolescence (ages 13-15 years) (Figure 1). I hypothesized that girls with higher neural activity to the anticipation of social rewards would show stronger positive associations between perceived social threat in daily life at baseline and social anxiety symptom severity at two-year follow-up (controlling for baseline social anxiety). This aligns with neurobiological susceptibility to social context models (Richey et al., 2019; Schriber & Guyer, 2016), with heightened neural activity to reward anticipation serving as the susceptibility factor.

To fully align with a differential susceptibility model, though, it needs to be shown that youth with higher neural reactivity to reward anticipation not only develop the most severe social anxiety symptoms when they perceive high social threat, but also develop the least severe social anxiety symptoms when they perceive low social threat (i.e., there is evidence of a significant cross-over interaction). If no cross-over interaction exists, and/or the susceptibility factor (neural
reactivity to social reward anticipation) is significantly associated with the predictor variable (daily social threat) or outcome (Wave 2 social anxiety symptom severity), the interaction aligns more with a dual-risk or diathesis-stress model (Belsky et al., 2007). The diathesis-stress model, which was originally created to explain schizophrenia (Rosenthal, 1970; Walker & Diforio, 1997), predicts that neurobiologically vulnerable individuals are disproportionately negatively affected in adverse environments; this model does not predict disproportional benefit in positive environments. Thus, diathesis-stress models predict an ordinal interaction rather than a disordinal or cross-over interaction.

I hypothesized that findings would align with a differential susceptibility model, such that youth with higher neural activity to the anticipation of social rewards would be the most susceptible to both negative and positive environments. More specifically, I hypothesized that youth with high neural activity would develop the most severe social anxiety symptoms at two-year follow-up when they reported high daily social threat at baseline and the least severe symptoms when they reported the lowest daily social threat at baseline.

I also examined specificity of this model to social anxiety (Aim 2) using a series of sensitivity analyses that 1) adjusted for depressive symptoms in the primary social anxiety model, 2) tested depressive symptoms as the outcome in the model, and 3) tested generalized anxiety symptoms as the outcome in the model. Controlling for depressive symptoms is particularly important in this work because altered neural reward function is associated with depressive symptoms in adolescence (Forbes & Dahl, 2012). It is critical to test whether associations between neural reward function and social anxiety can be better explained by symptoms of depression that frequently co-occur with social anxiety. I hypothesized that the model would be specific to social
anxiety (even when controlling for depressive symptoms) and would not generalize to predict generalized anxiety or depressive symptoms.

Finally, I examined specificity of this model to reward anticipation by replacing neural activation to social reward anticipation with neural activation to social *punishment* anticipation in the primary (social anxiety) model. I hypothesized that the model would generalize to neural activation to punishment anticipation. This aligns with prior research showing that youth at temperamental risk for anxiety are sensitive to incentives more generally, not only rewards specifically (e.g., Bar-Haim et al., 2009; Guyer et al., 2006).

Though not depicted in the figure, all models controlled for pubertal status. Pubertal status is important to consider when studying brain development and social anxiety in adolescence. As discussed in Section 1.2.1, the significant hormonal changes occurring during puberty are likely to influence the developing brain, including the mPFC. Developmental models also 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 (Blakemore, 2008; Nelson et al., 2005; Silk et al., 2012a). Early adolescent girls more advanced in pubertal status may also be at higher risk for peer victimization (Troop-Gordon, 2017) and social anxiety (Blumenthal et al., 2011; Deardorff et al., 2007; Kaltiala-Heino, 2003). Given the effects of pubertal maturation on function in social-affective neural regions and associations between puberty and social stress and anxiety, pubertal status was included in all models.
Figure 1. Proposed model. NAcc = nucleus accumbens, Caud = caudate, AI = anterior insula, BLA = basolateral amygdala, Thal = thalamus; dACC = dorsal anterior cingulate cortex; Social threat is measured using ecological momentary assessment.
2.0 Methods

2.1 Participants

One-hundred-twenty-nine early adolescent girls ages 11 to 13 were recruited for participation in this longitudinal study via online advertisements and announcements in the community. See Table 1 for demographic and clinical characteristics. Girls were recruited based on parent-reported sex at birth; gender identity was not assessed at recruitment. This study oversampled for shy/fearful temperament, a risk factor for the development of social anxiety in adolescence and adulthood (Sandstrom et al., 2020). Temperament was assessed prior to participants’ first visit using the Early Adolescent Temperament Questionnaire- Revised (EATQ-R; Ellis & Rothbart, 2001). The EATQ-R was designed to measure temperament traits in early adolescence (ages 9-15), with items specific to adolescent life experiences. To determine temperament status, participants were compared against established distribution scores of the EATQ-R shyness and fear scales (Ellis & Rothbart, 2001). The sample was stratified such that approximately 2/3 of participants (n=85) scored > 0.75 SDs above the mean on the parent- or adolescent-rated fear scales (3.12 for parent-report, 3.48 for adolescent-report) or shyness scales (2.99 for parent-report, 3.16 for adolescent-report). All other participants (n=44) scored below this cut-off and were considered to be in the normative range of shy/fearful temperament.

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), obsessive-compulsive disorder, post-traumatic stress disorder, major depressive disorder, or any psychotic or autism spectrum disorder, as determined by the Kiddie-Schedule for Affective Disorders and
Schizophrenia (K-SADS-PL; Kaufman, Birmaher, Axelson, Perepletchikova, Brent & Ryan, 2016). In addition, participants 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, the presence of any MRI contraindications, presence of head injury or congenital neurological anomalies (based on parent report), acute suicidality, taking medications that affect the central nervous system (e.g., selective serotonin reuptake inhibitors), and ocular conditions that would impede eye tracking measurement and/or ability to see clearly without prescription glasses. Stimulants were permitted if use was discontinued for 36 hours prior to the scan.

All participants were included in analyses; missing data were estimated using full-information maximum likelihood procedures. For completeness, though, we report reasons for missing data for the primary variables in the model. First, fMRI data were available for 87 participants. Reasons for missing fMRI data included: 1) Excess movement (N=25), 2) Scan not completed (N=9), 3) Missing data (N=7), or 4) An incidental finding that impeded analyses (N=1). Usable EMA data were available for 105 participants. EMA data were missing due to: 1) Low completion rates (< 25%; N=4), 2) Data quality issue (i.e., random responding; N=2), 3) Less than 3 negative interactions with peers (N=11), 4) EMA dropout or study withdrawal (N=6), or 5) Technical problem (no data collected; N=1). Wave 1 social anxiety symptom data were available for 126 girls; three girls had missing data due to issues with administration (i.e., the questionnaire was administered incorrectly by the study diagnostician). Wave 2 social anxiety symptom data were available for 117 girls; 12 girls had missing data because they dropped out from the study prior to data collection. Girls with missing data did not significantly differ from girls with full data on any of the measures included in the final analysis (p's>.10).
<table>
<thead>
<tr>
<th>Table 1. Key demographic and clinical characteristics of the sample</th>
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<tbody>
<tr>
<td><strong>Wave 1 (Baseline)</strong></td>
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<tr>
<td><strong>n (%)</strong></td>
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<tr>
<td>Age</td>
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<tr>
<td>Pubertal status (average score)</td>
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<tr>
<td>Total family income</td>
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<tr>
<td><strong>Diagnosis (Current)</strong></td>
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<tr>
<td>Specific phobia</td>
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<tr>
<td>Attention-deficit/hyperactivity disorder</td>
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<tr>
<td>Predominately inattentive</td>
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<tr>
<td>Combined type</td>
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<tr>
<td>Unspecified</td>
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<tr>
<td>Oppositional defiant disorder</td>
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<tr>
<td>Unspecified disruptive behavior disorder</td>
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<td>Tic disorder</td>
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<td>Enuresis</td>
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<td><strong>Wave 2 (Two-Year Follow-Up)</strong></td>
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<tr>
<td>Age</td>
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<tr>
<td>Pubertal status (average score)</td>
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<tr>
<td>Total family income</td>
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<tr>
<td><strong>Diagnosis (Current)</strong></td>
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<tr>
<td>Major depressive disorder</td>
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<td>Persistent depressive disorder</td>
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<td>Anxiety disorders</td>
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<td>Social anxiety disorder</td>
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<td>Specific phobia</td>
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<td>Combined type</td>
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<tr>
<td>Unspecified</td>
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<tr>
<td>Oppositional defiant disorder</td>
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<tr>
<td>Unspecified disruptive behavior disorder</td>
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<tr>
<td><strong>Diagnosis (Past; i.e., between Wave 1 and Wave 2)</strong></td>
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<tr>
<td>Major depressive disorder</td>
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<tr>
<td>Adjustment disorder with depressed mood</td>
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<tr>
<td>Unspecified depressive disorder</td>
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<tr>
<td>Anxiety disorders</td>
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<tr>
<td>Social anxiety disorder</td>
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<tr>
<td>Generalized anxiety disorder</td>
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<tr>
<td>Specific phobia</td>
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<tr>
<td>Unspecified anxiety disorder</td>
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<tr>
<td>Oppositional defiant disorder</td>
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<tr>
<td><strong>Race/Ethnicity</strong></td>
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<td>Black/African-American</td>
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<tr>
<td>Asian</td>
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<tr>
<td>Biracial</td>
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<tr>
<td>Native American</td>
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<tr>
<td>Other</td>
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</tbody>
</table>

27
Hispanic or Latino | 10 (7.8%)

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. Parents provided informed consent and youth provide informed assent to acknowledge their voluntary agreement to participant in the research. Data were collected from multiple laboratory visits conducted over a three-year period between 2016 and 2021.

Following informed consent (Wave 1), a research assistant administered the WASI to participants and a clinical interviewer (a master’s level graduate student or doctoral level therapist) administered the K-SADS-PL to determine eligibility and completed the Liebowitz Social Anxiety Scale for Children and Adolescents (LSAS-CA; Masia et al., 1999) for a measure of adolescent social anxiety symptoms. During a follow-up visit to the lab, approximately two weeks after the initial visit, participants were given an android smartphone to complete an 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. Approximately two weeks later, youth completed the Peer Social Incentive Delay (P-SID) task at the University of Pittsburgh Magnetic Resonance Research Center (MRRC). Approximately two years after the initial visit (Wave 2), the LSAS-CA was re-administered to measure social anxiety symptoms.
2.2.1 Ecological Momentary Assessment (EMA) Protocol

Data on real-world social threat experiences were collected using cell-phone EMA at Wave 1. Youth were given a pre-programmed android smartphone on which they entered 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 phones, participants were asked to answer questions about their most recent social interactions and their emotional responses to these interactions for 16 consecutive days. 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 54 observations. This large number of samples allows for a more stable estimate of “typical functioning,” even in the potential presence of several atypical days. Compliance in this sample was 81.3% (SD = 13.9%, range = 37.0% - 100%). These questions took approximately five minutes to complete at each interval.

2.2.2 FMRI Acquisition

Before entering the real MRI scanner at both Wave 1 and Wave 2, participants were trained in a simulation MRI scanner (“mock scanner”) to familiarize them to the tight space and the loud sounds of the scanner. Scanning took place on the same 3T Siemens Prisma magnet at both time points. 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 on their right hands; all participants included in analyses were right handed.

Anatomical images covering the entire brain were acquired first using a three-dimension magnetization-prepared rapid gradient-echo T1-weighted sequence (repetition time [TR]=2300ms, echo time [TE]=3.93ms, flip angle 9°, inversion time [TI]=900ms, voxel size=1 mm³). Functional scans were preceded by a localizer. Functional images were acquired using multi-band gradient echo-planar (EPI) sequences (60 slices, three-factor multiband) sensitive to BOLD contrast [T2*] (TR=1500ms, TE=30ms, flip angle 55°, voxel size=2.3 x 2.3 x 2.3 mm). Field maps were acquired using gradient echo planar imaging sequence for correction of field distortions in the functional images with the following parameters: TR=590ms, TE1=4.92ms, TE2=7.38ms, voxel size=2.3 x 2.3 x 2.3 mm, flip angle 60°. Following this scan, the Peer Social Incentive Delay (P-SID) task was administered in the scanner.

2.3 Measures

2.3.1 Social Anxiety Symptoms

The Liebowitz Social Anxiety Scale for Children and Adolescents (LSAS-CA; Masia et al., 1999) is a clinician-rating scale used to measure social anxiety symptoms in children and adolescents. The measure consists of 24 items, 12 social interaction situations (e.g., “looking at people you don’t know well in the eyes”) and 12 performance situations (e.g., “asking questions in class”). The clinician reads the list of 24 social situations to each adolescent and their participating parent and asks the adolescent to rate how anxious each situation made them over the
past week on a Likert scale of 0 (not at all) to 3 (very much). The adolescent is also asked to rate how much they tried to avoid the situation using the same 0 to 3 scale. Parents are asked to provide their input, and the clinician can adjust the adolescent’s ratings based on parent input, clinical judgment, and direct behavioral observations. The LSAS-CA provides seven scores: (1) anxiety related to social interaction, (2) performance anxiety, (3) total anxiety, (4) avoidance of social interaction, (5) avoidance of performance situations, (6) total avoidance, and (7) a total LSAS-CA score. The total LSAS-CA score was used in the main analyses for the present study, though exploratory analyses also included the LSAS total anxiety score (LSAS Anxiety) and LSAS total avoidance score (LSAS Avoid). For the LSAS-CA total score, a cutoff of 22.5 represents the best balance of sensitivity and specificity when distinguishing between individuals with social anxiety disorder and non-anxious individuals, whereas a cutoff of 29.5 is optimal for distinguishing social anxiety disorder from other anxiety disorders (Masia-Warner et al., 2003). In the present study, internal consistency of the LSAS-CA was high at baseline ($\alpha = .94$) and two-year follow-up ($\alpha = .96$).

2.3.2 Social Threat EMA Measure

To assess youths’ perceptions of social threat in daily life, we used the prompt: “Think about the interaction with other kids your age that made you feel the worst since the last beep.” They were asked to type out details about this interaction. If participants could not think of a negative interaction, they could select an option that states, “I am having trouble thinking of something.” They were then probed with follow-up questions to help them think about what happened since the last beep (e.g., “What were you doing when you completed the last beep?”; “Was there anything minor that happened that bugged you, like somebody said or did something
that annoyed you, hurt your feelings just a little, or disappointed you?”). If participants continued to indicate that they did not have a negative interaction, this observation was coded as “no negative interaction.” Participants were then given a checklist that included statements that described 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 (Figure 2). Examples of social threat statements include, “I felt criticized,” and “I felt disliked or rejected.”

<table>
<thead>
<tr>
<th>Prompt: Check any statements that describe what you were thinking or feeling during the interaction (check all that apply):</th>
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<tbody>
<tr>
<td>[] I worried about what someone thought of me</td>
</tr>
<tr>
<td>[] I was afraid someone didn’t like me</td>
</tr>
<tr>
<td>[] I was embarrassed</td>
</tr>
<tr>
<td>[] I felt criticized</td>
</tr>
<tr>
<td>[] I was worried that I would say or do the wrong thing</td>
</tr>
<tr>
<td>[] I felt left out or ignored</td>
</tr>
<tr>
<td>[] I found it hard to talk with someone</td>
</tr>
<tr>
<td>[] I felt disliked or rejected</td>
</tr>
</tbody>
</table>

Figure 2. EMA social threat items.

2.3.3 Peer Social Incentive Delay (P-SID) Task

The Peer Social Incentive Delay (P-SID) task (Kaurin et al., 2022) is a social adaptation of the original Monetary Incentive Delay task (MID; Knutson et al., 2000), and was designed to measure brain activity related to social rewards and punishments. A novel “peer observation” version of the task was created to examine neural activation to social feedback from a virtual peer. At a laboratory visit prior to the scan, participants viewed fictional photos and autobiographical
profiles (including hobbies and personality traits) of age-matched girls whom they were told were participating in the study at other institutions. Participants were asked to select and rank which girls they would most like to interact with during the MRI scan.

At the start of the fMRI visit, participants were told that they were matched with two of the girls they ranked highly (first and second) at their last visit. Participants were told that these girls were participating in the study at other sites and that they would be interacting with these girls during the fMRI tasks. Further, they were told that one of the girls would be watching the participant complete the P-SID fMRI task and providing feedback after each trial by sending a smiling, frowning, or neutral (blurry) picture of themselves based on the participant’s performance. To increase believability, participants were also asked to view (via a mock video feed) and evaluate this peer’s performance on the P-SID task prior to completing the fMRI task themselves. The peer’s performance on the P-SID task was computer-generated.

Participants interacted with these virtual peers first during the Chatroom Interact task in the MRI scanner (Silk et al., 2014); this was done to increase the participant’s familiarity with the peers (and increase believability) prior to the P-SID task. Participants completed the P-SID task immediately following the Chatroom task. The P-SID task (Figure 3) consists of one run of 72 trials (27 social reward, 27 social punishment, 18 control). Each trial proceeded in the following order: cue (500 ms), fixation cross (1500-3500 ms), target slide (500 ms), blank screen (1000 ms), peer feedback (1650 ms), and blank screen (2500-5000 ms). Participants were instructed to press a button with their index finger as quickly as possible when a target (white square) appeared on the screen. The target slide was always presented for 500ms but target presentation on that slide was variable (160-500 ms) to ensure that hit rates in different conditions were similar across participants. At the start of each trial, a cue (circle, square, or triangle) signaled the possible
outcomes when the participant pressed the button fast enough (i.e., response fell within the target presentation time) or was too slow. In the social reward condition, a circle signaled possible positive feedback (peer’s happy face) for a fast response or neutral feedback (peer’s blurry face) for a slow response. In the social punishment condition, a square signaled negative feedback (peer’s angry face) for a slow response or neutral feedback (peer’s blurry face) for a fast response. In the control condition, a triangle cued a neutral outcome (peer’s blurry face) regardless of performance. Total duration of the P-SID task was 12 minutes 2 sec (480 volumes). For the primary analysis, we examined neural activity during social reward anticipation (circle reward cue) relative to neutral anticipation (triangle cue). In sensitivity analyses, we examined neural activity during social punishment (vs. neutral) anticipation.

Figure 3. P-SID task schematic. (A) An initial cue (500ms) and fixation cross (1500-3500ms) were first presented for each trial. Participants were instructed to press a button as fast as possible when a target (white square) appeared on the screen. Following the target, a black screen was displayed (1000ms) and the feedback was presented (1650ms). A second black screen followed the feedback prior to the next trial (2500-5000ms). (B) The task consisted of 27 trials (27 social reward, 25 social punishment, 18 control).
2.3.4 Measures Used for Sensitivity Analyses and Covariates

2.3.4.1 Pubertal Status

At Wave 1, participants 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 good internal consistency (median alpha coefficient = .77; Petersen et al., 1988). 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, these include breast development, growth spurt, and menarche (associated with gonadal hormones) and pubic/body hair and skin changes (associated with adrenal hormones; Shirtcliff, Dahl, & Pollak, 2009). The total score (combining changes associated with gonadal and adrenal hormones) was used as a proxy of pubertal status in the current study.

2.3.4.2 Generalized Anxiety Symptoms

At each wave, participants completed a modified (44-item) version of the Screen for Anxiety and Related Emotional Disorders-Child version (SCARED; Birmaher et al., 1997). The SCARED is a self-report checklist that assesses multiple symptoms of anxiety across several domains – generalized anxiety, social anxiety, school avoidance, panic symptoms, and separation anxiety. Sensitivity analyses for the current study used the generalized anxiety subscale score. The generalized anxiety subscale includes nine items. Example items include, “I am a worrier,” and “People tell me that I worry too much”. In the present sample, the generalized anxiety subscale of
the SCARED demonstrated acceptable internal consistency at baseline ($\alpha = .82$) and two-year follow-up ($\alpha = .81$).

2.3.4.3 Depressive Symptoms

At each time point of the current study, participants completed the 33-item Mood and Feelings Questionnaire (MFQ)-Child Version (Angold & Costello, 1987) to assess depressive symptoms (e.g., mood, appetite, sleep, psychomotor functioning, inappropriate guilt and feelings of worthlessness, suicidal ideation) over the past two-week period. Each item on the MFQ is rated on a three-point scale (0=not true, 1=sometimes true, 2=true) and summed to create a total score. Scores range from 0-66, with higher scores indicating greater depressive symptoms. In the present sample, the MFQ demonstrated high internal consistency at baseline ($\alpha = .92$) and two-year follow-up ($\alpha = .94$).

2.4 Analytic Plan

2.4.1 FMRI Data Preprocessing and Analysis

Statistical Parametric Mapping software (SPM12; Wellcome Trust Centre for Neuroimaging, UK) was used to preprocess functional images. Preprocessing included: 1) Reorientation of anatomical and functional images to the anterior and posterior commissure line, 2) Use of the FieldMap toolbox to create a voxel displacement map (VDM) for distortion correction of the functional images, 3) Use of the Realign and Unwarp procedure to generate motion parameter files and correct for distortion using the VDM, 4) Registration of functional
images to the anatomical image, 5) Segmentation of anatomical images into gray and white matter maps using the International Consortium for Human Brain Mapping (ICBM) tissue probability maps, 6) Registration of anatomical and functional images to MNI space using the ICBM152 template with 2mm voxels, 7) Smoothing of normalized images using a \(6mm^3\) full-width at half-maximum gaussian kernel, and 8) Repair of motion artifacts using ArtRepair (Mazaika et al. 2007). Scans with >0.5 mm of incremental motion, >3mm from the baseline image, and/or 3 standard deviations [SD] intensity shifts were considered outliers; outlier scans were replaced with a linear interpolation between the two nearest non-outlier scans. Participants with >25% of volumes with excess movement (i.e., outliers) were excluded.

For the first-level analyses, individual effects were estimated using the general linear model (GLM) approach implemented in SPM12. For the P-SID task, we modeled anticipation trials (i.e., cues), social reward feedback (i.e., smiling face following reward cue), social punishment feedback (i.e., angry face following punishment cue), social reward miss feedback (blurry face following reward cue), social punishment hit feedback (blurry face following punishment cue), and neutral feedback (blurry face following neutral cue) at the first level, with motion parameters included as nuisance regressors.

Group-level analyses focused on several ROIs: the caudate head, caudate body, putamen, NAcc, anterior insula (AI), basolateral amygdala (BLA), precuneus, dorsal ACC, and mediodorsal nucleus (MDN) of the thalamus. The dACC, putamen, AI, and precuneus ROIs were constructed using the Brainnetome Atlas (http://www.brainnetome.org/). The caudate body, caudate head, and mediodorsal nucleus of the thalamus were defined using the Talaraich atlas in WFU Pick Atlas, and the NAcc was defined using the IBASPM71 atlas in Pick Atlas. The BLA was defined in Pick Atlas as a 3.5 mm sphere centered at \((x=-26, y=-5, z=-23)\) for left BLA; \(x=29, y=-3, z=-23\) for right
BLA), as in previous studies on these region (e.g., Gao et al., 2021). All ROIs were bilateral; unilateral ROIs were combined using the FSL -maths function. ROIs are displayed in Figure 4 below. The decision to focus on these ROIs is informed by prior research on neural activation to social reward anticipation in adolescents (Rademacher et al., 2010; Martins et al., 2021). Average parameter estimates were extracted from each ROI using the MarsBar toolbox for SPM12.

Figure 4. Regions-of-interest used in analyses. (A) precuneus (yellow) and dACC (red); (B) BLA; (C) putamen (green), NAcc (white), caudate body (copper), caudate head (red), AI (blue) (red); (D) MDN (blue).
2.4.2 Social Threat EMA Analysis

Social threat sum scores (i.e., the sum of the social threat statements endorsed for each negative peer experience) were used in analyses. Items were summed across each observation because we assumed each item to be weighted equally, without the possibility of missing data within each observation because the items were administered in a checkbox format. Previous research using multilevel exploratory factor analysis has shown that these eight social threat items load on a one factor solution at both the within- and between-person level (Sequeira et al., 2021). These social threat scores can be aggregated across time to create one measure of average social threat for each participant; previous research has shown that this is a reliable and valid measure of social threat (Sequeira et al., 2021).

2.4.3 Analytic Plan

IBM SPSS Version 26 was used to evaluate descriptive statistics for observed variables, changes in anxiety symptoms over time, and correlations between observed variables. The remaining analyses were conducted using Mplus version 7 (Muthén & Muthén, 1998-2015). First, a latent factor of neural activation to social reward (vs. neutral) anticipation was created using exploratory factor analysis (EFA). The formation of a latent variable allowed us to examine how a correlated social reward neural network, which includes the caudate (body and head), putamen, NAcc, anterior insula, basolateral amygdala, thalamus, dorsal ACC, and precuneus, is associated with behavior. Factor loadings of .40 and above were considered significant; modification indices were considered to improve model fit and the model was confirmed using confirmatory factor analysis.
An EFA was chosen as a first step rather than a CFA because it was highly possible that the EFA would yield several different, equally plausible and theoretically-sound results. First, all regions could load significantly on one factor of social reward anticipation. A second possibility was that two factors may arise: one “reward” factor potentially consisting of the caudate, putamen, NAcc, amygdala, and thalamus and one “social salience” factor potentially consisting of the AI, dorsal ACC, and precuneus. A third possibility was that three or more factors would arise; in this case, I decided a priori to consider the factor loadings, prior research, and theory to identify which factors to include in the study. Should multiple factors arise, I also decided a priori to test the full model first with all factors included and then with each factor included in separate models. If no coherent factor or factors arose from the EFA, I would instead run the model with the NAcc alone, as this region is most consistently engaged during social and non-social reward anticipation.

The proposed model (Aim 1; Figure 1) was then tested using structural equation modeling (SEM) in Mplus. The model was estimated using full information maximum likelihood (Enders & Bandalos, 2001) and the robust maximum likelihood (MLR) estimator, which features robust standard errors. The model was first tested without the interaction term (i.e., with main effects of daily social threat, the latent neural social reward factor, pubertal status, baseline social anxiety symptoms, and number of negative interactions) to determine model fit.

Five fit statistics were used to evaluate overall fit of the measurement and structural models: the chi-square ($\chi^2$) statistic, Comparative Fit Index (CFI), the Tucker-Lewis Index (TLI), the Root Mean Square Error of Approximation (RMSEA), and the Standardized Root Mean Square Residual (SRMR). Conventional cut-off criteria proposed by Hu and Bentler (1999) were used to assess model fit: RMSEA < 0.06, CFI > 0.95, TLI > 0.95, and SRMR < 0.08. The $\chi^2$ tests the hypothesis that the hypothesized covariance matrix differs from the observed covariance matrix.
Thus, a non-significant $\chi^2$ is indicative of good model fit. Because absolute fit indices are not estimated when a random slope is added to the model (as is done when estimating an interaction with a latent variable), relative fit indices (i.e., Bayesian information criterion, BIC) and the Satorra-Bentler (2001) scaled chi-square different test (using loglikelihood values) were used to compare models with and without the interaction term (i.e., the interaction between the latent neural social reward factor and daily social threat).

I used SEM to test how interactions between heightened neural reactivity to social rewards (Wave 1) and experiences of social threat (Wave 1) in early adolescent girls oversampled for shy or fearful temperament contribute to social anxiety symptoms in mid-adolescence (Wave 2) (Aim 1). Social anxiety symptoms at Wave 1 were covaried on Wave 2 symptoms. The total number of negative interactions with peers was also covaried on Wave 2 symptoms to isolate how the quality of negative peer interactions contributes to social anxiety symptoms through interactions with reward brain function, above and beyond the quantity of negative interactions. Pubertal status was covaried on both Wave 2 social anxiety symptoms and the neural social reward latent factor.

Significant interactions were probed using simple slopes analysis in Mplus. The Johnson-Neyman technique was used to compute regions of significance for the moderator; this is a standard approach for determining values of the moderator for which associations between the predictor variable and outcome are significant. Significant interactions were then probed further to determine whether findings align with a differential susceptibility model (Belsky et al., 2007). Specifically, for significant interactions, I computed tests of the regions of significance on X (the predictor). As explained by Roisman et al. (2012), the test of the regions of significance on X (RoS on X) determines the range of the predictor variable for which the moderator and the outcome variable are significantly associated with each other (typically bounded by +/- 2 SD from the mean.
of the predictor variable). Results are consistent with a differential susceptibility hypothesis if the association between the moderator (neural activity) and outcome variable (social anxiety symptoms) is significant at both high and low ends of the range of the predictor variable (i.e., within +/- 2 SDs of daily social threat). Results are more consistent with a diathesis-stress model if the association between the moderator (neural activity) and outcome variable (social anxiety symptoms) is significant at the high end but not the low end of the predictor variable (daily social threat). To provide additional support for a differential susceptibility model, Roisman et al. (2012) also suggest reporting the proportion of the interaction (PoI) index, which represents the proportion of the total interaction that is represented on the left and right of the crossover point. PoI values between .40 and .60 (ideally near .50) are consistent with a differential susceptibility model. To plot the interactions and generate RoS on X and PoI values, a web-based application developed by R. Chris Fraley was used (https://www.yourpersonality.net/interaction/). Predictor variables were standardized prior to creation of interaction plots using this application to aid in interpretability.

A series of sensitivity analyses were also run to test specificity of the model. First, I tested how interactions between neural social reward function and daily social threat predict symptoms of generalized anxiety or depression to test specificity to social anxiety (Aim 2). The model was also run with both social anxiety symptoms and depressive symptoms as the outcome variables to examine whether reward function is more strongly associated with depressive symptoms that might co-occur with anxiety, in contrast to core symptoms of anxiety. Finally, the model was run replacing neural activity to social reward anticipation with neural activity to social punishment anticipation. This allowed me to test whether social anxiety is associated with reward anticipation specifically or incentive anticipation more generally (Aim 3).
3.0 Results

3.1 Preliminary Results

Descriptive statistics and intercorrelations between observed variables, calculated using SPSS version 26, can be found in Table 2. In the full sample with complete symptom data (N=114), total LSAS scores increased significantly from Wave 1 to Wave 2 (t(113)=2.10, p=.038). This was driven by an increase in scores on the avoidance subscale of the LSAS, corresponding to how often individuals have avoided feared situations over the past two weeks (t(113)=2.94, p=.004). Scores on the anxiety subscale of the LSAS, corresponding to how anxious or fearful different situations have made the teen feel over the past two weeks, did not increase significantly (t(113)=1.02, p=.308). Depressive symptoms did not significantly increase from Wave 1 to Wave 2 (t(116)=1.44, p=.152). Generalized anxiety symptoms did increase significantly from Wave 1 to Wave 2 (t(113)=2.35, p=.021).

Social anxiety severity (total LSAS scores) at Wave 2 (two-year follow-up) was measured during the COVID-19 pandemic (March 2020 – December 2020) for 20% of the sample (N=26); for the remainder of the sample, Wave 2 symptom severity was measured prior to the COVID-19 pandemic. Social anxiety severity at Wave 2 differed modestly but non-significantly (Welch t(1, 38)=3.00, p=.092) between girls with symptoms measured during the COVID-19 pandemic (M=36.04, SD=25.04) and girls with two-year symptoms measured pre-pandemic (M=26.41, SD=23.12). Of note, however, these groups also differed in social anxiety symptom severity measured at baseline (Welch t(1, 36)=4.42, p=.043), such that higher social anxiety severity at baseline (Wave 1) was seen for girls with two-year (Wave 2) symptoms measured during the
COVID-19 pandemic (M=31.46, SD=20.33) than girls with two-year symptoms measured pre-pandemic (M=22.27, SD=16.82). Social anxiety symptom severity increased from baseline to two-year follow up in both groups, though this increase was not significant in either group, likely due to a decrease in power from splitting the sample (pre-pandemic group: \(t(87)=1.94, p=.055\), pandemic group: \(t(25)=.84, p=.407\)). These groups (girls with Wave 2 symptoms measured pre-pandemic or during the pandemic) did not significantly differ in age, pubertal status, or risk type (\(ps>.60\)).

Youth high in shy/fearful temperament did not significantly differ from youth low to moderate in shy/fearful temperament in neural activity to social reward vs. neutral anticipation in any ROI (\(ps>.25\)). These temperament groups also did not show differences in daily social threat (\(F(1,104)=.01, p=.911\)). As expected, these groups differed significantly in social anxiety symptom severity at baseline (\(F(1,125)=17.85, p<.001\)) and at two-year follow up (\(F(1,116)=6.40, p=.013\)), such that youth recruited to be higher in shy/fearful temperament had higher social anxiety severity at both time points.
## Table 2. Descriptive statistics and intercorrelations between observed variables

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<td>-.39</td>
<td>-.68</td>
<td>.30</td>
<td>3.37</td>
<td>.50</td>
<td>-.39</td>
<td>.52</td>
<td>3.08</td>
<td>.17</td>
<td>.55</td>
<td>.46</td>
<td>.96</td>
<td>.68</td>
<td>-.11</td>
<td>.44</td>
</tr>
</tbody>
</table>


Note. Bolded values indicate $p < .05$; activity for all brain regions is for the contrast social reward vs. neutral anticipation; PDS = Pubertal Development Scale, sx = symptoms, GAD = Generalized Anxiety Disorder, MDN = mediodorsal nucleus of the thalamus, ACC = anterior cingulate cortex. Age is included in this table for descriptive purposes but was not used in any analyses.
3.2 Neural Social Reward Latent Factor

Eighty-seven participants had usable fMRI data and were included in the EFA. With nine variables included in the model, the minimum amount of data recommended for factor analysis (10 participants per variable) was not satisfied. All data were inspected closely prior to the EFA; no regions exhibited skewness values > 2, and only the BLA showed potentially meaningful kurtosis (3.08) due to the presence of two “extreme outliers” (i.e., data points in the third quartile + 3*interquartile range or in the first quartile – 3*interquartile range). There was no reason to assume that these data points were errors in the dataset, thus they were left as is. However, it should be noted that none of the results of the EFA changed when these outliers were winsorized.

All EFA models were estimated in Mplus using an oblique Geomin rotation (the default rotation criterion for EFA) because of its ideal balancing of interpretability and factor complexity (Browne, 2001; Sass & Schmitt, 2010). The optimal number of factors was determined through consideration of a parallel analysis (Horn, 1965) and interpretability of the resulting factors. The eigenvalues of the estimated correlation matrix exceeded the random data generated eigenvalues for only the first factor (first three empirical eigenvalues = 5.26, 0.96, 0.83; first three random data eigenvalues = 1.52, 1.34, 1.21).

Though a one-factor model was suggested, model fit was poor ($\chi^2$ = 97.78, $df$ = 27, $p < .001$, RMSEA = .17, CFI = .86, TLI = .82, SRMR = .06). A two-factor solution significantly improved model fit ($\Delta \chi^2$ = 56.0, $\Delta df$ = 8, $p < .001$) but was not indicated by the parallel analysis and the factors were not theoretically sound. A three-factor solution failed to converge. Examining factor loadings for the one-factor solution revealed relatively low loading of BLA activity (.32) relative to other
variables (NAcc=.51, AI=.76, Caudate Body=.86, Caudate Head=.75, MDN=.86, Precuneus=.66, Putamen=.89, dACC=.81). The decision was made a priori to remove variables with factor loadings below 0.4. Modification indices were also considered to improve model fit; only modifications that would contribute to a chi-square change larger than 10 and were theoretically sound were considered. Modification indices indicated that adding a correlation between the two regions of the caudate would contribute to a chi-square change of 15 and that adding a correlation between the dACC and AI would contribute to a chi-square change of 39. These correlations were sensible from a theoretical standpoint; one would expect that activation in the head and body of the caudate would be highly correlated, and the dACC and AI have strong structural connections and often coactivate functionally in affective salience tasks (Ghaziri et al., 2017).

A confirmatory factor analysis was run to confirm this modified model, which removed the BLA and added correlations added between the dACC and AI, as well as between the two caudate regions. Variance of the NAcc factor was constrained to 1 to fix the scale of the latent factor, as this was chosen a priori as the most representative ROI of the network. Model fit for this final neural social reward latent factor was good ($\chi^2=25.54$, $df=18$, $p=.111$; RMSEA=.069, CFI=.99, TLI=.98, SRMR=.038).

### 3.3 Aim 1: Test of the Structural Model Predicting Wave 2 Social Anxiety Symptoms

The structural model included the resulting latent factor of neural social reward function (with all regions except for the BLA included) and daily social threat data, and controlled for pubertal status, baseline social anxiety symptom severity, and the total number of negative peer interactions. Daily social threat data were not included for seven participants because these
participants reported less than three negative interactions over the EMA collection period, consistent with previous work using this measure (Sequeira et al., 2021). Data were assumed to be missing at random; girls with missing EMA, fMRI, or questionnaire data did not differ from girls with complete data on any of the variables included in the study (ps>.10). Thus, missing data were handled using FIML as planned.

The model was first estimated without the interaction term to examine model fit. This model included 105 participants with 48 free parameters; 24 participants were not included because of missing daily social threat data, which could not be estimated in the full model due to its interaction with the latent factor. To better compare models with and without the interaction term, these participants were also excluded from the model without the interaction term. This restricted model showed good fit to the data ($\chi^2=62.64$, $df=56$, $p=.253$; RMSEA=.034, CFI=.98, TLI=.98, SRMR=.073; BIC=5217.60). In this model, significant main effects were seen for baseline social anxiety symptom severity ($\beta=.39; B=.52, SE(B)=.14, p<.001$) and number of negative peer interactions ($\beta=-.17; B=-.42, SE(B)=.19, p=.029$). Controlling for all other variables in the model, girls who reported higher social anxiety symptom severity at baseline and less frequent negative peer interactions at baseline had more severe clinician-rated social anxiety symptoms at two-year follow-up. No significant main effects emerged for daily social threat ($\beta=.08; B=2.51, SE(B)=2.41, p=.297$), pubertal status ($\beta=-.05; B=-1.15, SE(B)=1.90, p=.544$), or the neural social reward latent factor ($\beta=.11; B=3.32, SE(B)=4.11, p=.419$).

To test Aim 1, the interaction between daily social threat and the latent neural social reward factor was then added to the model. Daily social threat data were centered prior to the creation of the interaction term in Mplus. The full analysis included 105 observations with 49 free parameters; as previously stated, 24 participants were excluded due to missing daily social threat data. Again,
this model was severely underpowered, which should be considered when interpreting the following findings. Though absolute fit statistics (i.e., Chi-square, CFI/TLI, SRMR, RMSEA) are not available for this type of model (these statistics are not estimated once random slopes are added to the model), BIC increased slightly when adding the interaction term (BIC=5222.30, ΔBIC=+4.70), suggesting that adding the interaction term to the model did not significantly improve model fit. This was confirmed using the Satorra-Bentler scaled chi-square difference test, which is appropriate for models using an MLR estimator. This test revealed that adding the interaction to the model failed to significantly improve model fit (chi-square difference score=.05, df=1, p>.95). Unsurprisingly, then, the interaction term was not significant (B=-.02, SE(B)=3.87, p=.996) and explained almost no additional variance (<0.001%) in two-year social anxiety symptom severity. Similar to the restricted model, the only significant predictors of two-year social anxiety symptom severity were baseline social anxiety symptom severity (B=0.52, SE=.14, p<.001) and the total number of negative peer interactions (B=-.42, SE=.19, p=.030). Results for this model are detailed in Figure 5.

Findings were comparable using the LSAS Avoid subscale or the LSAS Anxiety subscale as the dependent variable. Findings were consistent when adjusting for depressive symptoms, risk type, or COVID-19 collection period (whether data were collected during the COVID-19 pandemic, dummy coded). No significant main effects of, or interactions between, the neural social reward latent factor and daily social threat emerged when restricting the sample to only participants with Wave 2 social anxiety symptoms measured prior to the COVID-19 pandemic (N=80).

Of note, all of these models were severely underpowered. Many experts recommend using the ratio of observations (participants) to estimated parameters (N:q) to estimate power. The
recommended $N:q$ ratio ranges from as low as 5 to 1 (Bentler & Chou, 1987) to as high as 20 to 1 (Kline, 2015). For these models, the ratio was around 2 to 1.

Figure 5. Results from the full structural equation model.
3.4 Exploratory Analysis: BLA Activation

A post-hoc analysis tested the interaction between neural activity and daily social threat separately for BLA activation to social reward vs. neutral anticipation, as this was the only region not included in the neural social reward latent factor. FIML was implemented by estimating the variances for the observed exogenous variables in the Model command. The MLR estimator was retained given the non-normal distribution of BLA activity (Table 2). Daily social threat and BLA activity were centered prior to the creation of the interaction term in Mplus.

In this just-identified \( (df=0) \) path model \( (N=129, \text{35 free parameters}; \text{model } R^2=.24, \text{SE}=.08, p=.002) \), the interaction between BLA activation to social reward (vs. neutral) anticipation and daily social threat was significant \( (\beta=.24; \text{B}=4.08, \text{SE(B)}=1.71, p=.017) \). Including this interaction in the model explained an additional 5% of the variance in social anxiety symptom severity (without the interaction term, model \( R^2=.19, \text{SE}=.07, p=.007 \)). As displayed in Table 3, main effects of baseline social anxiety symptoms \( (\beta=.33; \text{B}=.43, \text{SE(B)}=.13, p=.001) \) and number of negative peer interactions \( (\beta=-.20; \text{B}=-.47, \text{SE(B)}=.19, p=.016) \) on two-year social anxiety symptom severity were also seen. A significant main effect of BLA activation also emerged \( (\beta=.20; \text{B}=2.71, \text{SE(B)}=1.36, p=.047) \) but is not interpreted due to the presence of a significant interaction. Removing the interaction term from the model, BLA activation had a moderate but non-significant effect on Wave 2 social anxiety symptoms \( (\beta=.22; \text{B}=2.85, \text{SE(B)}=1.58, p=.070) \).

The interaction between daily social threat and BLA activation to social reward (vs. neutral) anticipation was probed using simple slopes analysis in Mplus. This analysis revealed that the simple slope for the effect of daily social threat on two-year follow-up social anxiety severity was only significant at high levels \( (+1 \text{ SD}) \) of BLA activation to social reward vs. neutral anticipation \( (\text{B}=11.30, \text{SE}=4.68, p=.016; \text{Table 3}) \). Johnson-Neyman analysis in Mplus further
showed that the effect of daily social threat on two-year social anxiety symptom severity was significant only at BLA values above .30 SDs above the mean.

To test whether this interaction aligned with a differential susceptibility model, regions of significance on X (RoS on X) tests (Roisman et al., 2012) were then conducted to identify whether BLA activity (the moderator) and social anxiety symptoms (the outcome variable) were significantly associated at the low and/or high ends of the predictor variable (daily social threat). Figure 6 shows the association between daily social threat and social anxiety symptoms moderated by low (-1SD) and high (+1SD) levels of BLA activation to social reward (vs. neutral) anticipation. The area shaded in gray refers to regions where the two slopes are significantly different. As shown in Figure 6, no significant cross-over interaction emerged; only at high levels of daily social threat were BLA activity and social anxiety symptoms significantly associated. Moreover, girls with high levels of daily social threat (+1 SD above the mean) and high levels of BLA activity (+1SD above the mean) were at the highest risk for social anxiety symptoms at two-year follow-up.

Findings were comparable using the LSAS Avoid subscale or the LSAS Anxiety subscale as the dependent variable. The interaction between BLA activation to social reward vs. neutral anticipation and daily social threat predicting total social anxiety symptoms remained significant when the sample was restricted to only participants with social anxiety symptoms collected prior to the COVID-19 pandemic (N=99; interaction B=7.03, SE=2.94, p=.017). The interaction between BLA activation and daily social threat also remained significant when running the model using list-wise deletion (interaction B=3.37, SE=1.37, p=.014), which restricted the sample to N=68 with full data.
Table 3. Full model results for BLA activation to social reward (vs. neutral) anticipation and daily social threat predicting social anxiety, generalized anxiety, and depressive symptoms

<table>
<thead>
<tr>
<th>Model DV: Social Anxiety Symptoms</th>
<th>$\beta$</th>
<th>B</th>
<th>SE(B)</th>
<th>p-value (B)</th>
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<tr>
<td>Intercept</td>
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<td>36.23</td>
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<td>Pubertal status</td>
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<td>-2.93</td>
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<td>.108</td>
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<td>Baseline social anxiety severity</td>
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<td>.13</td>
<td>.001</td>
</tr>
<tr>
<td>Number of negative peer interactions</td>
<td>-.20</td>
<td>-.47</td>
<td>.19</td>
<td>.016</td>
</tr>
<tr>
<td>Daily social threat</td>
<td>.13</td>
<td>2.53</td>
<td>1.58</td>
<td>.115</td>
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<td>BLA reactivity to social reward (vs. neutral) anticipation</td>
<td>.20</td>
<td>1.36</td>
<td>1.99</td>
<td>.047</td>
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<td><strong>BLA reactivity X daily social threat</strong></td>
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<td><strong>4.08</strong></td>
<td><strong>1.71</strong></td>
<td><strong>.017</strong></td>
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<td>Simple slope at low levels of BLA activity (-1 SD)</td>
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<td>3.61</td>
<td>.169</td>
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<td>Simple slope at moderate levels of BLA activity (Mean)</td>
<td>3.99</td>
<td>2.53</td>
<td>.115</td>
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<tr>
<td>Simple slope at high levels of BLA activity (+1 SD)</td>
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<th>SE(B)</th>
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<td>Baseline generalized anxiety severity</td>
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<td>.006</td>
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<td>Number of negative peer interactions</td>
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<td>.03</td>
<td>.350</td>
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<td>Daily social threat</td>
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<td>.47</td>
<td>.146</td>
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<td>.00</td>
<td>.22</td>
<td>.998</td>
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<td><strong>BLA reactivity X daily social threat</strong></td>
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<td>.47</td>
<td>.146</td>
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<td>Simple slope at high levels of BLA activity (+1 SD)</td>
<td><strong>2.23</strong></td>
<td><strong>.68</strong></td>
<td><strong>.001</strong></td>
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<table>
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<th>SE(B)</th>
<th>p-value (B)</th>
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<td>Pubertal status</td>
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<td>-.69</td>
<td>.83</td>
<td>.982</td>
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<tr>
<td>Baseline depressive severity</td>
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<td>.14</td>
<td>.003</td>
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<td>Number of negative peer interactions</td>
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<td>.00</td>
<td>.11</td>
<td>.982</td>
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<td>1.67</td>
<td>.001</td>
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<tr>
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<td>.03</td>
<td>.59</td>
<td>.955</td>
</tr>
<tr>
<td><strong>BLA reactivity X daily social threat</strong></td>
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<td><strong>1.94</strong></td>
<td><strong>.84</strong></td>
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<td>1.67</td>
<td>.001</td>
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<tr>
<td>Simple slope at high levels of BLA activity (+1 SD)</td>
<td>9.55</td>
<td>2.84</td>
<td>.001</td>
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</table>

Note. Not depicted in this table are means, variances, and correlations of and between independent variables, which were estimated in the model and contribute to the total number of free parameters ($N=35$ for each model). DV = dependent variable.
3.4.1 Aim 2: Specificity to Social Anxiety Symptoms

Sensitivity analyses were run to examine whether interactions between BLA activation to social reward vs. neutral anticipation and daily social threat also predicted two-year follow-up (Wave 2) generalized anxiety symptoms or depressive symptoms. Results can be found in Table 3. Interactions between BLA activity and daily social threat at baseline significantly predicted both self-reported generalized anxiety symptoms (interaction $\beta=.27$, $B=.72$, $SE(B)=.25$, $p=.004$; model $R^2=.19$, $SE=.07$, $p=.005$) and depressive symptoms (interaction $\beta=.25$, $B=1.94$, $SE(B)=.84$, $p=.004$).
two years later (controlling for baseline symptoms, pubertal status, and total number of negative interactions). In the depressive symptoms model only, daily social threat also had a significant main effect on two-year follow-up depressive symptoms ($\beta = .40, p = .001$). Johnson-Neyman analyses revealed that the effect of daily social threat on two-year follow-up generalized anxiety symptoms was significant when BLA activity was above .3 SDs above the mean. The effect of daily social threat on two-year depressive symptoms was significant when BLA activity was above .6 SDs below the mean.

In line with a differential susceptibility model (Belsky et al., 2007), significant cross-over interactions emerged for both generalized anxiety symptom and depressive symptom models. Figures 7 and 8 show that at both high and low levels of daily social threat (within the range of -2 SD to +2 SD), significant differences between low (-1 SD) and high (+1 SD) BLA activity were found. The PoI was .50 for the generalized anxiety symptom model and .51 for the depressive symptom model, which supports a differential susceptibility model. Moreover, in these models, BLA reactivity to social reward (vs. neutral) feedback, the suggested susceptibility factor, was not significantly associated with Wave 2 generalized anxiety or depressive symptoms (estimated $rs < .02, ps > .87$) or daily social threat (estimated $|r|s < .17, ps > .15$), which are necessary conditions to support a differential susceptibility hypothesis (Belsky et al., 2007).

The interaction between BLA activity and daily social threat also predicted social anxiety symptoms when controlling for depressive symptoms ($\beta = .24; B = 4.08, SE = 1.70, p = .016$). Further, when depressive symptoms and social anxiety symptoms were both included as outcomes in the model, the interaction significantly predicted both depressive symptoms ($\beta = .25, SE(\beta) = .10, p = .012$) and social anxiety symptoms ($\beta = .12, SE(\beta) = .05, p = .027$). However, model fit for this latter model was poor, likely due to the small sample size and high number of parameter estimates.
being estimated ($\chi^2=9.42$, $df=5$, $p=.093$; RMSEA=.08, CFI=.92, TLI=.76, SRMR=.041); thus, this finding should be interpreted with great caution. Social anxiety symptoms and depressive symptoms were moderately correlated in this model ($r=.32$, $p<.001$), suggesting some but not full overlap.

Figure 7. Results: BLA and generalized anxiety. Effect of daily social threat on two-year follow-up generalized anxiety symptoms at high (+1 SD) and low (-1 SD) levels of BLA activation to social reward (vs. neutral) anticipation. Predictors were standardized prior to the formation of this plot; social threat scores are plotted from -2SD to 2SD with a mean of 0. *$p<.05$
3.4.2 Aim 3: Specificity to Social Reward

To explore specificity to neural social reward function, sensitivity analyses were also conducted to examine whether the model replicated for BLA activation to social punishment vs. neutral anticipation. BLA activation to social reward vs. neutral anticipation and BLA activation to social punishment vs. neutral anticipation were significantly correlated ($r=.53, p<.001$). However, no interaction emerged between BLA activation to social punishment vs. neutral
anticipation and daily social threat (β=.05; B=1.10, SE(B)=2.15, p=.607). Additionally, no main effect of daily social threat was found (β=.02; B=.55, SE(B)=2.48, p=.824), though a significant main effect of BLA activation to social punishment vs. neutral anticipation was seen (β=.25; B=3.78, SE(B)=1.63, p=.020). The only other significant main effect to emerge in this model was baseline social anxiety symptom severity (β=.34; B=.45, SE(B)=.13, p<.001).
4.0 Discussion

Over the past decade, the positive valence system (PVS), including neural reward function, has been well-studied in relation to the development and treatment of depression during adolescence. Despite strong comorbidity between anxiety and depression, and significant increases in rates of anxiety disorders during adolescence, the PVS is often left out of conversations regarding the etiology or treatment of anxiety disorders. However, emerging research suggests that in studying the development of anxiety disorders during adolescence, greater attention be paid to the role of the PVS, and neural reward function specifically. Particularly relevant is consistent research showing altered neural reward function in youth at temperamental risk for social anxiety disorder (e.g., Bar-Haim et al., 2009; Guyer et al., 2006). These findings have contributed to novel developmental theories and models postulating that neural reward function plays a key role in the development of social anxiety disorder through interactions with social stress (Richey et al., 2019; Sequeira et al., 2022). These models hold promise for better understanding how and why social anxiety increases substantially during the adolescent period, which could have important influences on efforts to intervene with those most at risk for this often debilitating and highly distressing disorder.

The goal of this study was to test a core component of these developmental models linking neural reward function and social stress to social anxiety symptom development in adolescence (Richey et al., 2019; Sequeira et al., 2022). The sample was limited to adolescent girls, given increased risk for social anxiety disorder during adolescence (Merikangas et al., 2010). Taking a multimethod approach linking fMRI and EMA, the first aim of this study was to test neural reactivity to reward anticipation as an individual-level factor making youth more susceptible to
social threat in their environments. I hypothesized that girls with higher neural reactivity to the anticipation of socially rewarding (vs. neutral) feedback would show stronger associations between daily perceptions of social threat from peers and social anxiety symptoms two years later. Findings partially support this hypothesis, such that the association between social threat in daily life and social anxiety symptoms was stronger for girls with higher basolateral amygdala (BLA) activation to social reward vs. neutral anticipation. Findings were specific to the BLA region-of-interest; the main analysis linking daily social threat and activity in a neural social reward latent factor to social anxiety symptoms did not yield significant findings. The second aim of this study was to examine specificity to social anxiety symptoms, and the final aim was to examine specificity to neural reward (vs. threat) function. Though specificity to neural reward function was found, the interaction between BLA activity and daily social threat was not specific to increases in social anxiety symptoms but extended to predict both generalized anxiety symptoms and depressive symptoms. Implications of these findings and limitations of this study will now be discussed.

4.1 Aim 1: Interactions between Neural Reward Function and Daily Social Threat Predict Social Anxiety Symptoms in Adolescence

In testing the full structural model linking activity in the neural social reward latent factor and daily social threat to two-year follow-up social anxiety symptom severity, no main effects of, or interactions between, the neural social reward latent factor and daily social threat emerged. Null findings could be related to the high number of parameters and low number of observations included in this model, which limited power to detect significant effects; this is discussed further in Section 4.3. Unexpectedly, a main effect of number of negative peer interactions on social
anxiety symptom severity was seen, such that youth reporting fewer negative peer interactions at baseline had more severe clinician-rated social anxiety symptoms two years later. One possibility is that girls reporting less frequent negative interactions with peers were spending less time with their peers overall (i.e., greater social avoidance), which could be a risk factor for social anxiety. However, this finding should be interpreted with caution as some girls may have had less frequent negative interactions only because they responded to fewer EMA prompts. Thus, the number of negative peer interactions measure is not an ideal measure of frequency of peer stress in daily life but was instead included in analyses as a covariate.

4.1.1 Post-hoc Analysis: BLA Activity

A post-hoc analysis tested the proposed model with BLA activation to social reward (vs. neutral) anticipation as the moderator, as this was the only region that did not load significantly on the neural social reward latent factor. When testing the BLA as the moderator, the primary hypothesis was supported, such that BLA activation to social reward anticipation significantly interacted with daily social threat to predict social anxiety symptoms two years later. As hypothesized, only at high levels of BLA activity was the positive association between daily social threat and two-year social anxiety symptom severity significant.

Contrary to hypotheses, though, a significant cross-over interaction was not observed, failing to fully align with the neurobiological susceptibility to social context hypothesis (and a differential susceptibility model). At low levels of daily social threat, social anxiety symptoms were relatively low regardless of BLA reactivity. Only at high levels of social threat were differences in BLA activity associated with differences in social anxiety symptoms, such that girls with high levels of BLA activity developed more severe symptoms than girls with low levels of
BLA activity. This finding is more in line with a diathesis-stress model than a differential susceptibility model. Like the differential susceptibility model, the diathesis-stress model emphasizes the importance of considering individual-level and interpersonal risk factors for psychopathology and can be useful for explaining why only some individuals with certain neurobiological vulnerabilities develop symptoms of psychopathology. Unlike the differential susceptibility model, the diathesis-stress model does not form hypotheses about how individuals function in positive, supportive environments; rather, this model focuses only on how stressful environments exacerbate negative outcomes for individuals with certain underlying vulnerabilities (or diatheses).

Present findings could suggest that high BLA activation to social reward (vs. neutral) anticipation is a neurobiological vulnerability factor that makes adolescents more susceptible to the negative effects of social threat. Conceptually, the BLA is a region that activates in response to reward-predictive cues during learning to support learning from reward contingencies (Lichtenberg et al., 2017; Wassum et al., 2011, 2015). Higher daily social threat may thus impact reinforcement learning processes more strongly in individuals with high BLA activity to social reward cues. Altered reinforcement learning processes resulting from interactions between high BLA activity and high social threat could lead individuals to expect more negative social feedback in their environments, contributing to more severe social anxiety symptoms over time. Thinking back to Julie, our client in the introduction, high sensitivity to contingencies in the environment that have a high potential to be socially rewarding (e.g., waiting to be asked to dance at a school dance) may be detrimental when time and time again, she is not asked to dance and feels left out and rejected. Over time, Julie may come to associate potentially rewarding situations with failure, and come to avoid events that could signal potential social reward and/or view them through a
distorted lens, supporting the development of social anxiety disorder. This interpretation aligns well with Richey and colleagues’ (2019) Sensitivity Shift Theory. It must be noted, however, that the PSID task is not a reinforcement learning task, thus interpreting present findings in the context of reward learning remains speculative.

Present findings are interesting to consider in relation to previous work from Jarcho and colleagues (2019), who found that for youth (age 11 years) experiencing high peer victimization, higher early childhood wariness (measured at ages 2–7 years) and higher amygdala reactivity to unpredictably positive peer feedback (age 11) were associated with more severe social anxiety symptoms at age 11. Like the current study, this previous study suggests that for youth at temperamental risk for social anxiety, social stress and reward-related amygdala activity are important to consider in relation to social anxiety symptoms. Moreover, the authors interpret these findings using a diathesis-stress model, such that neural responsivity to unexpected positive social feedback exacerbates the link between peer victimization and social anxiety symptoms in youth showing high levels of early childhood wariness. However, the task used by Jarcho and colleagues differed substantially from the PSID task, amygdala activity was specific to the receipt of unpredictable social reward, and the size of this study was small (N=47); similarities and differences in findings should thus be interpreted with caution.

There are several potential explanations for why the interaction between BLA activity and daily social threat on social anxiety symptoms failed to align with a differential susceptibility model. First, low levels of daily social threat do not necessarily imply high levels of daily social reward, which could help explain the lack of a cross-over interaction. It is still possible that girls with high levels of BLA reactivity would thrive in environments marked by high levels of social reward (e.g., feeling socially accepted, high frequency of positive social feedback), as they may
come to expect positive social feedback over time. This would be interesting to explore in future work. Relatedly, varying interpretations of low values on the social threat scale could help explain the absence of a cross-over interaction. The daily social threat measure is not an objective measure of peer rejection. Rather, it is a measure of emotional reactivity in negative social situations, tapping youths’ perceptions of how socially threatened (e.g., criticized, embarrassed, rejected) they feel in negative interactions with peers. While low levels of daily social threat could represent a positive social environment, very low levels of social threat could also index blunted reactivity in negative social situations and/or low awareness of social cues and social experiences. Importantly, girls who are relatively unaffected by negative peer interactions or show low social awareness are unlikely to be highly socially anxious, as social anxiety is marked by an intense and persistent fear of negative evaluation. Thus, girls reporting low levels of daily social threat may be at the lowest risk for social anxiety symptoms regardless of brain activity, which could help explain the present pattern of findings.

Finally, failure to capture a significant cross-over interaction could be related to the relatively small sample size of the current study (Del Giudice, 2017; Roisman et al., 2012). Because statistical significance is affected by sample size, larger studies might be more likely to detect significantly different slopes at both the low and high ends of the predictor variable (thus supporting a differential susceptibility hypothesis) than smaller studies. However, it is also the case that in larger studies, even small evidence for a crossover interaction at the highest or lowest value of the predictor would be consistent with a differential susceptibility hypothesis, even if this has very limited theoretical significance (Roisman et al., 2012).
4.1.1.1 Aims 2 & 3: Specificity

Contrary to hypotheses, present findings were specific to BLA activation to social *reward* anticipation, such that the model did not replicate for BLA activation to social *punishment* activation. Interestingly, though, a main effect of BLA activation to punishment vs. neutral anticipation on two-year follow-up social anxiety symptoms was found, contributing to a large literature showing that heightened amygdala reactivity to potential threat may be one risk factor for the development of anxiety disorders (Shackman et al., 2016).

Also contrary to hypotheses, specificity to social anxiety symptoms was not supported. Interactions between BLA activation to social reward anticipation and daily social threat predicted both generalized anxiety symptoms and depressive symptoms at two-year follow-up. Only at moderate to high levels of BLA activity did significant positive associations between daily social threat and generalized anxiety or depressive symptoms emerge. Thus, the interaction between neural social reward function and social threat may be relevant for disorders broadly associated with altered functioning in the positive valence system domain and may help us understand deficits in positive affect, anhedonia, and motivation seen transdiagnostically. However, it is also possible that interactions between neural social reward function and social threat predict altered functioning in the negative valence domain (e.g., fear, hypersensitivity to threat, higher negative affect), which could also explain generalization of the model to depressive symptoms and generalized anxiety symptoms. Including more transdiagnostic outcomes in future work could help clarify these findings.

Interestingly, cross-over interactions between BLA activity and daily social threat were observed for both generalized anxiety and depressive symptom models. Aligning with a neurobiological susceptibility to social context hypothesis (and differential susceptibility model),
girls with high BLA activation to social reward (vs. neutral) anticipation reported the most severe
generalized anxiety and depressive symptoms at Wave 2 when they perceived high levels of social
threat and reported the least severe generalized anxiety and depressive symptoms when they
perceived the lowest levels of social threat. In these generalized anxiety and depression models,
high BLA activity could be viewed as a neurobiological factor that makes youth more susceptible
to both negative and positive social contexts. This contrasts with the social anxiety model, in which
high BLA activity was interpreted as a neurobiological vulnerability factor leading to
disproportionately unfavorable outcomes in negative social contexts specifically.

Given the strong overlap between symptoms of social anxiety, generalized anxiety, and
depression, it is interesting to consider why these findings may be diverging. One possibility is
that the mechanisms linking BLA reactivity and daily social threat to generalized anxiety and
depression differ from those linking BLA reactivity and daily social threat to social anxiety
symptoms. For example, interactions between BLA reactivity and daily social threat may impact
social anxiety symptoms but not generalized anxiety or depressive symptoms through effects on
reward learning, or vice-versa. More research would be needed to understand whether and how
these mechanisms are truly diverging, as present findings cannot yet speak to this.

Interpreting findings for the generalized anxiety and depression models in the context of a
differential susceptibility hypothesis assumes that low social threat is indexing a more positive
social environment, which may be the case. However, it could also be that case that very low levels
of perceived social threat are indicative of lower social awareness, as previously discussed in the
context of social anxiety. Moreover, while low social awareness may be protective against social
anxiety, this may not be the case for depression or generalized anxiety. For example, girls with
lower social awareness may have difficulty making friends and “fitting in”, which could contribute
to loneliness and anhedonia, as well as anxiety around not fitting in. This could be particularly problematic in combination with reduced BLA reactivity to social reward anticipation, as this pattern of brain activity could impede learning from any positive social interactions that do occur, potentially contributing to lower motivation to seek out socially rewarding experiences. Thus, at low levels of daily social threat, girls with reduced BLA reactivity to social reward anticipation may be more socially disengaged and disconnected, and therefore at higher risk for depression and generalized anxiety, than youth with increased BLA activity (who may be better able to learn from positive social interactions and more motivated to engage socially). These potential explanations, though speculative, underscore the nuance needed when interpreting (what appear to be) differential susceptibility models. Labeling girls with reduced neural reactivity to social reward anticipation only as “fixed” and unaffected by their social environments, as is proposed by traditional differential susceptibility models, may fail to capture the complex processes actually occurring.

It is also interesting to note that a small-moderate main effect of BLA reactivity to social reward vs. neutral anticipation emerged only in the social anxiety model. This is consistent with a diathesis-stress model, as it suggests that high BLA reactivity to reward anticipation could itself be a risk factor for social anxiety, but even more so in the presence of high daily social threat. This is also consistent with prior research showing heightened neural reactivity to reward anticipation in youth with, or at risk for, social anxiety disorder (e.g., Bar-Haim et al., 2009; Guyer et al., 2006). For a differential susceptibility model to be supported, the susceptibility factor and outcome must be independent (Belsky et al., 2007), which was true for the generalized anxiety and depressive symptoms models. This finding could further support the hypothesis that different mechanisms are at play linking BLA activity and daily social threat to the development of social anxiety relative
to generalized anxiety and depression. However, it should also be noted that the present study employed a sample of girls at temperamental risk for social anxiety specifically; it is unclear whether and how present findings are related to the unique nature of this sample and how findings might generalize to girls not recruited on the basis of risk for social anxiety disorder.

Different patterns of findings for generalized anxiety and depressive symptoms relative to social anxiety symptoms could also be related to the relatively small sample size and resulting low power across these analyses. Larger, more well-powered studies may be able to better differentiate whether interactions between reward function and social stress predict internalizing psychopathology according to a differential susceptibility or diathesis-stress model. Notably, for the depressive symptom model, significant differences in BLA activity were only seen at extremely high and low levels of daily social threat, potentially calling into question the theoretical relevance of this cross-over interaction.

Finally, different patterns of findings could be related to differences in measurement of anxiety and depression symptoms. Generalized anxiety and depressive symptoms were self-reported and social anxiety symptoms were clinician-rated. Daily social threat scores were significantly correlated with Wave 1 and Wave 2 depressive symptoms and generalized anxiety symptoms, but were not significantly correlated with Wave 1 or 2 social anxiety symptoms, suggesting that shared method variance could play a role in biasing the generalized anxiety and depressive symptom models. Similarities and differences in the mechanisms linking interactions between social threat and neural reward function to symptoms of social anxiety, generalized anxiety, and depression could be interesting for future research to explore in greater depth.

Generalization of findings to symptoms of depression and generalized anxiety suggest that future work may benefit from taking more of a transdiagnostic approach to studying how brain-
environment interactions influence the development of clinically-relevant constructs that cut across psychiatric disorders. This aligns with efforts put forth by RDoC (Insel et al., 2010) and HiTOP (Kotov et al., 2017). A more dimensional approach could also be taken in future research focused on understanding mechanisms underlying social anxiety specifically. Like all psychiatric disorders, SAD is a multifaceted construct with many core components, including fear of negative evaluation, social avoidance, and physiological symptoms in response to perceived threat. Studying the development of social anxiety using a group approach (i.e., comparing youth with and without social anxiety disorder) or a continuous approach that sums all social anxiety symptoms masks the ability to tease apart whether different etiological mechanisms are related to different components of SAD. In the current sample, increases in LSAS scores from baseline to two-year follow-up appeared to be driven by increases in avoidance of social interactions, rather than anxiety. Though interactions between BLA activation to social reward (vs. neutral) anticipation and daily social threat predicted both LSAS avoidance scores and anxiety scores, this may not always be the case. Including more transdiagnostic symptoms and behaviors as outcomes in future work may help us identify more specific etiological mechanisms, and ultimately more specific targets for intervention.

4.2 Neural Social Reward Factor: An SEM Approach to Modeling Brain Function

A novel contribution of this study was testing a latent factor of neural social reward function. Latent variable modeling allows for examining associations between a latent construct, which is unmeasurable, and true observations (Borsboom et al., 2003). Latent variables are created to explain why observed (measured) variables are related; this approach assumes that multiple
observed variables correlate with each other because they are caused by the same latent variable. Nine regions (i.e., the BLA, AI, dACC, MDN, NAcc, ventral caudate, dorsal caudate, putamen, and precuneus) were included in an exploratory factor analysis (EFA) and follow-up confirmatory factor analysis (CFA). All regions except for the BLA were found to load acceptably on one factor, termed the neural social reward latent factor. This approach to modeling brain activity is still relatively uncommon, though some previous work has used an SEM approach (typically a CFA) to model brain structure and function (e.g., Baskin-Sommers, Neumann, Copy, & Kiehl, 2016; Bolt et al., 2018; Kim-Spoon et al., 2021; Kurkela et al., 2022; Lahey et al., 2012).

There are many benefits of creating a latent variable to measure brain function, including improvements in reliability (see Cooper, Jackson, Barch, & Braver, 2019 for a more thorough discussion of such benefits). Task-based fMRI has been overwhelmed with concerns regarding reliability; in the same sample, task-based fMRI can yield reliable results for one brain region and unreliable results for three others. With SEM, the unexplained variance (error) is modeled separately from the shared variance, which removes error from the latent variable. Since latent variables are capturing the “true” variance, they are often considered error-free and reliable (Cooper et al., 2019). Creating a latent factor of a brain network may thus be a more reliable approach than relying on single ROIs as predictors. Moreover, reducing measurement error with a latent variable yields greater statistical power for hypothesis testing, relative to testing independent ROIs (Lahey et al., 2012). A latent variable approach, and more broadly an SEM approach, also allows for the creation and testing of flexible and complex models.

While there are clear benefits of a latent variable and SEM approach, there are also important drawbacks. One potential reason why this approach has not been implemented more in fMRI research is that latent variable models require large samples; SEM incurs high degrees of
freedom due to the high number of parameters. Such large samples are not often seen in fMRI studies (Poldrack et al., 2017), largely due to high costs of running MRI scans. Overfitting the latent variable model is also a very real possibility, especially because SEM is so flexible. Researchers may be tempted to overfit the model according to proposed modification indices; in the current study, only modification indices that were theoretically indicated were considered.

Though a strength of the latent variable approach to modeling brain activity is the acknowledgement that brain regions function in a network, not in isolation, another downside of this approach is the loss of sensitivity gained at the individual ROI level. Interpretation is more difficult when including multiple brain regions in a latent factor, as it is unclear exactly what this factor represents. In other words, loading all of these regions onto one latent factor of neural social reward function nicely acknowledges the interdependence between these regions while potentially sacrificing specificity and interpretability.

In creating the neural social reward latent factor, an EFA was originally chosen over a CFA because the regions tested are traditionally considered in separate networks, though all regions play a role in processing social stimuli and/or reward-related stimuli. The NAcc, caudate, and putamen are all regions of the striatum and work together to integrate reward-related information and support reward learning and decision-making (Delgado, 2007). The dACC and AI are cortical hubs of the salience network (Seeley, 2019) and have a direct structural connection, supporting their frequent functional co-activation (Ghaziri et al., 2017). These regions have also been considered part of a “social pain” neural network, though more recent research suggests that these regions also activate to socially rewarding stimuli, and thus may better be conceptualized as responding to socially salient information (e.g., Dalgleish et al., 2017; Sequeira et al., 2021). Beyond its hypothesized role in processing socially salient information and reward-based-
decision-making (Bush et al., 2002), the dACC is also considered a region of the cognitive control network due to its role in error detection (Holroyd et al., 2004) and has been shown to play a role in fear expression (Milad et al., 2007). The mediodorsal nucleus of the thalamus similarly has been linked to a variety of tasks beyond processing rewards, including memory and learning, likely related to its unique positioning that allows it to form connections with many cerebral structures, including the prefrontal cortex and ACC (Alelú-Paz & Giménez-Amaya, 2008). The precuneus is a core region of the default-mode network and plays a crucial role in self-referential network (Utevsky, Smith, & Huettel, 2014).

Due to the diversity of these functions, it was hypothesized that the EFA could return multiple neural factors corresponding with different neural networks. However, this was not the case; all regions except for the BLA loaded acceptably onto one factor. This factor was modified and confirmed using a CFA approach. This result was not entirely surprising, given how extensive connections in the brain truly are. Even the precuneus, which is rarely considered part of the reward network, has widespread connections with other regions included in the social reward latent factor, including with the dorsolateral caudate nucleus, putamen, ACC, anterior insula, and thalamus (Cavanna & Trimble, 2006; Ghaziri et al., 2017). Moreover, though these regions may be considered key hubs of different networks, they were all chosen a priori due evidence that they play a role in processing social rewards.

Interestingly, the BLA did not load strongly onto any factor in the EFA, which could beg the question of how this region differs from the others. Across many studies, the BLA has been shown to play a key role in reward learning and decision-making, particularly through its interactions with the orbitofrontal cortex (OFC; Lichtenberg et al., 2017; Wassum et al., 2011; Wassum & Izquierdo, 2015), a region not included in the present study. Non-human animal
research has shown that connections between the BLA and OFC support cue-triggered reward expectations (Lichtenberg et al., 2017). The BLA also plays a role in learning and memory through connections with the hippocampus (Yang & Wang, 2017) and in associative learning and motivation through connections with the NAcc (Phillips, Ahn, & Howland, 2003; Wassum & Izquierdo, 2015). The BLA may thus play a more specific role in reward learning than other regions included in the neural social reward latent factor, the latter of which may be involved more broadly in processing socially rewarding information. This, however, remains speculative.

4.3 Limitations and Future Directions

Present findings should be viewed in light of some important limitations. First, as discussed, SEM requires large sample sizes to have statistical power, particularly when estimating many parameters. Including eight regions in the final latent factor led to a large number of parameters, for which we did not have an adequate sample size, and thus power to detect a significant effect was low. Therefore, significant findings for the BLA may be better attributed to greater power to detect a significant effect, rather than a meaningful difference between the BLA and other regions making up the latent social reward factor.

Second, though using EMA to measure daily social threat was a strength of this study, this measure also had limitations. As mentioned, there are different potential ways to interpret this social threat measure. For example, while higher daily social threat scores could be capturing more severe social stress in daily life, girls with higher social threat scores could also be perceiving even relatively neutral interactions with peers as very threatening. Including a more objective measure of peer stress in future work testing the model proposed in this dissertation could provide more
context with which to interpret present findings. However, this should not discount the value of the present social threat measure, which provides an important indicator of how threatening each individual perceives their social environment to be. In many ways, youths’ perceptions of social threat may be more meaningful to their development than an objective measure. Moreover, most prior work testing the neurobiological susceptibility to social context hypothesis also uses self-report questionnaires that elicit youths’ perceptions of their social relationships (e.g., parenting or peer relationships) (e.g., Rudolph et al., 2020, 2021). When interpreting neurobiological susceptibility to social context research, it is important to consider how the data are being collected and who the informants are.

Perhaps more importantly, low social threat scores may not be a good indicator of a positive social context. While environments that are less socially threatening could be seen as relatively more positive than environments that are more socially threatening, low daily social threat is not akin to high daily social reward (e.g., feeling good in positive interactions with peers). Moreover, perceiving some social threat in negative interactions with peers may actually be adaptive and helpful for adolescents tasked with navigating complex social environments. A more ideal test of differential susceptibility would include predictor and outcome variables that have values ranging from truly positive on one end to truly negative on the other end.

It should also be noted that interpreting BLA activity as a susceptibility factor rests on an assumption that functional neural activity is trait-like, at least in the short term. Concerns have been raised regarding the stability of neural activity, particularly when neural activity is assessed using contrasts (e.g., social reward > neutral; Elliott et al., 2020). Additionally, reliable brain-behavior correlations may require thousands of participants (Marek, Tervo-Clemmons, et al., 2022). Integrating within-person changes in brain activity in future work may be a more reliable
way to assess how different environmental stressors interact with brain function to predict psychopathology in adolescence.

Replicating present analyses in larger samples, possibly leveraging data from large, cross-institutional studies such as the ABCD study (Casey et al., 2018), will be important before firm conclusions can be drawn. Replicating present analyses in samples with a larger age range would also allow researchers to assess whether there may be a sensitive development period in which the effects of social stress are most potent. Interactions between high BLA activation to reward anticipation and perceived social threat may have particularly strong impacts on psychopathology during adolescence relative to other developmental periods, given evidence of heightened neural responses to reward anticipation (Galván, 2010) and heightened sensitivity to social evaluation (Somerville, 2013) in adolescence. Interactions between pubertal status, brain function, and daily social threat were not probed in the present study because of the small sample size but could be explored in future work. Notably, though, pubertal status was not significantly associated with two-year social anxiety symptom severity or the latent factor of neural social reward function in the present sample.

The mechanisms through which social threat and brain activity interact to support increases in social anxiety symptoms during adolescence remain unknown. Though the introduction explored several ways in which social stress might “get inside the brain” to influence social anxiety symptom development, none of these cellular mechanisms were tested directly. Moreover, neural activity was only measured at one time point; whether and how daily social threat changed patterns of neural activity over time remains to be tested. As previously discussed, one possibility could be that daily social threat interrupts reward learning processes more in youth with high BLA
activation to social reward anticipation, given this region’s role in reward learning. This remains an interesting question for future research to explore.

Finally, for one in five participants, social anxiety symptoms were assessed during the COVID-19 pandemic, a period of time that may have had meaningful impacts on their trajectories of social anxiety symptom development. The stress of the pandemic, severe social restrictions and decreased opportunities for in-person social exposure, and transitions to novel means of social interaction (e.g., Zoom) could have contributed to increases in social anxiety in girls. Alternatively, though, the pandemic could have contributed to short-term decreases in social anxiety, as many girls no longer had to participate in social interactions that made them nervous (e.g., sports, clubs, eating in front of others) and many could keep their cameras off during virtual schooling.

For the current sample, it is difficult to know how the COVID-19 pandemic influenced the development of social anxiety symptoms; girls who had follow-up symptom data collected during the pandemic had higher symptoms of social anxiety (on average) at follow-up and at baseline relative to girls with follow-up data collected pre-pandemic. Moreover, because the subsample of girls with follow-up symptoms assessed during the COVID-19 pandemic was significantly smaller than the subsample of girls with symptoms assessed pre-pandemic, it is challenging to directly compare groups. Controlling for COVID-19 group (i.e., data collected pre-pandemic or collected during the pandemic) or restricting the sample to only girls with data collected pre-pandemic did not change the pattern of findings. However, future researchers will need to think critically about how to incorporate the COVID-19 pandemic and associated stressors into our understanding of adolescent socioemotional development. This is only becoming more apparent as we learn that COVID-19 is not an acute stressor but will have lasting changes on the social and emotional health and well-being of individuals and families around the world.
4.4 Clinical Implications of Findings

Identifying environmental and neurobiological mechanisms that contribute to the development of psychopathology can help elucidate more precise and biologically-informed intervention tools and strategies. Results from this study suggest that perceiving higher daily social threat is particularly detrimental for youth high in neurobiological sensitivity to potential social rewards. It remains unknown, though, whether this measure of social threat actually corresponds with more severe peer stress and victimization (a more objective measure), or whether it is the perception of greater peer stress or greater reactivity to peer stress (independent of the objective severity of the peer stress) that is key for understanding the present findings. Answering this question could have important influences on targets for intervention. If objective social stress is indeed most important for understanding increases in social anxiety (and generalized anxiety and depressive) symptoms in those at neurobiological risk, strategies to improve peer relationships or build social skills in at-risk youth may be more important. If more negative perceptions of social stress (or greater emotional reactivity to social stress) is most important for understanding increases in anxiety and depressive symptoms, cognitive restructuring and emotion regulation skills may prove more useful for at-risk youth. Importantly, using fMRI to identify youth at high neurobiological risk who might benefit from interventions that target peer relationships is not currently feasible, given how time- and money-intensive fMRI is. Future research could ascertain reliable behavioral or clinical correlates of high neurobiological sensitivity to reward contingencies to more easily test and replicate the current model, as well as to help identify youth who might benefit most from interventions that target this neurobiological mechanism.

Future work examining how reward learning plays a role in linking heightened neural reactivity to reward anticipation and social threat to symptoms of social anxiety (and depression
and generalized anxiety) may also have implications for treatment. If reward learning does emerge as a crucial mechanism linking high neurobiological sensitivity and social threat to social anxiety symptoms, clinical interventions that target reinforcement learning in at-risk youth may be ideal. As discussed by Richey and colleagues (2019), mindfulness strategies could be particularly relevant here. Mindfulness practice involves being fully present and aware in the present moment without judgment. If aberrant reward learning mechanisms are (as hypothesized) leading youth who are highly sensitive to reward contingencies and perceiving more social threat to expect more negative social feedback, mindfulness may help these youth re-learn associations between social interactions and their potential for reward by enhancing attention to and processing of positive interactions and events in the environment (Richey et al., 2019). Realizing that social interactions can be pleasurable may then motivate youth to engage more socially, reducing risk for social anxiety disorder and depression. Interestingly, in a randomized controlled trial of Mindfulness-Based Cognitive Therapy for adults with a history of depression, Geschwind et al. (2011) showed that this therapy was associated with increases in positive affect and an increased ability to boost positive affect by engaging in pleasant activities. Mindfulness practices have been integrated into the curricula for many schools, demonstrating how mindfulness is one promising intervention strategy that can reach a significant portion of youth.

Ideally, research into neurobiological mechanisms supporting the development of psychopathology will contribute not only to more biologically-informed intervention targets, but also to efforts to maximize treatment efficacy by understanding who may be most likely to benefit from these biologically-informed interventions. One possibility is that the interaction between high neurobiological sensitivity to anticipated social rewards and daily social threat predicting social anxiety symptoms is most relevant for youth high in early life behaviorally inhibited (BI)
temperament, as proposed in Richey et al. (2019)’s Sensitivity Shift Theory. Thus, high BI youth may be most likely to benefit from interventions targeting these mechanisms. High BI, which can be identified in early life (including infancy and toddlerhood) and is characterized in part by negative emotional reactions to novelty, has been consistently linked to high neural reactivity to reward anticipation (Sequeira et al., 2022). High neural reactivity to reward anticipation could support heightened responsivity to salient information in the environment, which may underlie distress to novelty (Fox et al., 2021). Moreover, research has consistently found that children and adolescents with a history of high BI are at higher risk for social anxiety symptoms and behaviors if they also show attentional biases towards threatening faces on the dot probe task (see Fox et al., 2021 for a review). The combination of high neural reactivity to reward anticipation and attending to/perceiving more social threat in the environments may thus be key for understanding increases in social anxiety symptoms for high BI youth in particular. As prevention programs are currently being developed and tested for high BI children and their families (Chronis-Tuscano et al., 2022), continuing to study how neural reward function and other PVS constructs are implicated in the development of social anxiety in this population may have meaningful and timely impacts on intervention development and implementation.

Though I suggest that findings from the present dissertation may be most clinically relevant for high BI youth, it is important to note that the present sample should not be considered a high BI sample. Though shy and fearful temperament in adolescence was assessed in this study, previous research in over 1000 early adolescents has shown that Kagan’s temperamental construct of BI is significantly (though only moderately) associated with scores on the EATQ shyness scale but is not associated with scores on the EATQ fear scale (Muris & Meesters, 2009). Using both shyness and fearfulness scales in the current study to create temperament groups could thus mask
associations between these groups and BI. In addition, reliability of these EATQ scales in the current study was low, and parent-child agreement on this questionnaire is generally low (Muris & Meesters, 2009), suggesting that this method of measuring shy or fearful temperament was successful in its goal of oversampling for social anxiety symptoms for the current study but group analyses may not be as meaningful. This could help explain why youth high in shy or fearful temperament did not differ from youth lower in shy or fearful temperament in neural activity to the anticipation of social rewards.

More research replicating and extending present findings needs to be done before firm recommendations for clinical intervention are made. More broadly, however, findings suggest that neural reward function has relevance for understanding not only the development of depressive symptoms but also social anxiety and generalized anxiety symptoms. Research should continue exploring the role of neural reward function and other PVS constructs (e.g., positive affect, behavioral approach) in social and generalized anxiety symptoms (see Sequeira et al., 2022). This research should pay close attention to how PVS constructs are related to anxiety symptoms separate from co-occurring depressive symptoms. If indicated by the addition of more research, intervention strategies that more broadly target the PVS (e.g., behavioral activation and positive activity scheduling, practicing gratitude, savoring positive emotions, mindfulness; see Richey et al., 2019 for a review) could be useful to integrate more in treatments for youth high in anxiety symptoms.

Finally, results from this study highlight the mechanistic overlap supporting the development of social anxiety, generalized anxiety, and depression, which suggests not only taking a more transdiagnostic approach to research, but also taking a more transdiagnostic approach to treatment. Relying less on psychiatric nosologies to guide treatment plans and focusing more on
targeting different constructs that cut across disorders (e.g., sensitivity to social evaluative threat, worry in social situations) holds promise for improving our approach to clinical intervention (see Dalgleish, Black, Johnston, & Bevan, 2020 for a review). This approach has been utilized in some intervention programs for youth, such as *The Modular Approach to Therapy for Anxiety, Depression, Trauma, or Conduct Problems* (MATCH-ADTC) (Chorpita & Weisz, 2009). Strategies that target similar constructs across different disorders already exist; for example, cognitive restructuring is used to address unhelpful or inaccurate thoughts across disorders and exposures are used to decrease avoidance across disorders. Thus, a transdiagnostic approach to treatment does not require developing brand new clinical tools, but rather finding ways to administer these tools in more flexible ways to meet the unique needs of each client.
5.0 Conclusions

Guided by developmental theory, this dissertation sheds light on brain-environment associations that contribute to increases in social anxiety symptoms during adolescence, a sensitive period of development marked by significant neurobiological and social changes. In a large sample of adolescent girls, high basolateral amygdala reactivity to potential social rewards interacted with greater social threat in daily life to predict more severe social anxiety symptoms over a two-year period. This study is strengthened by the integration of multiple ecologically-valid methods and a longitudinal design that captured a large developmental window (ages 11 to 17), as well as the use of a measure of social anxiety symptoms assessed by a diagnostician, reducing shared method variance. This study is novel in its creation of a latent factor of neural social reward function, though power to detect a significant effect in models incorporating this latent factor was low.

For clients like Julie from our introduction, these findings provide greater context with which to think about the development of her social anxiety symptoms. However, there are undoubtedly many other biopsychosocial influences on social anxiety symptom development; social threat and neural reward function are not the only two. Nonetheless, present findings provide a starting point upon which future research can build on and replicate (or fail to replicate) findings. This model could (and should) be integrated with other factors believed to confer risk for social anxiety symptoms in adolescent girls. Future research could also integrate reward learning into this model, to test whether reward learning is indeed a mechanism contributing to increases in social anxiety symptoms during adolescence. Further, this model should be tested with different developmental periods and with adolescents varying in racial, ethnic, gender, and sexual identities.
Importantly, interactions between neural reward function and daily social threat were not specific to social anxiety symptoms but extended to predict two-year depressive symptoms and generalized anxiety symptoms. Thus, interactions between neural reward function and social threat may predict altered functioning in the positive valence domain, which cuts across different categorical diagnoses. Taking a more dimensional, transdiagnostic approach in future research may help clarify how brain-environment interactions support the development of clinically impairing symptoms, such as low approach motivation or anhedonia. This could have meaningful impacts on understanding the development of adolescent psychopathology and future treatment development.


