# Common and Distinct Neural Mechanisms of Fear Acquisition and Reversal in comorbid Autism with Social Anxiety and Social Anxiety Disorder uncomplicated by Autism

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## Abstract

Social Anxiety (SAD) increases in prevalence as children enter adolescence. Adolescents with Autism Spectrum Disorder (ASD) are diagnosed with comorbid SAD at higher rates than these individuals are diagnosed with other clinical disorders, including depression and other anxiety disorders. However, there is little research on whether the presentation and neural underpinning of comorbid SAD within the context of ASD is the same as SAD alone. Individual and diagnostic differences exist in neural and biological mechanisms of fear conditioning. Characterization of whether neural mechanisms of fear are different within ASD with comorbid SAD and SAD alone may better inform clinical treatments. Accordingly, the present study characterizes neural responses during a fear-inducing experiment, as measured by fMRI. Fifty-seven adolescents participated in this study, with adolescents with ASD and SAD (n=17), SAD alone (n=20), and typically developing adolescents (n=20). All participants completed two fear conditioning and reversal paradigms while completing an fMRI scan. The paradigm consisted of a Social condition and Nonsocial condition. An ANOVA for fear conditioning was conducted. Results revealed significant activation in the Inferior Temporal Gyrus (ITG) during fear conditioning. No between group differences were observed, but within-group differences indicated differential modulation of the ITG in the ASD with SAD group in the Social condition compared to the Nonsocial condition. The SAD group demonstrated differential activation between conditioning stimuli in the Nonsocial condition, but not in the Social condition. Results indicate that adolescents with ASD and SAD may display different neural mechanisms for acquiring fear compared to typically developing peers. Results have potential to inform treatment approaches.

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## General Audience Abstract

Social Anxiety Disorder (SAD) refers to extreme fear of negative evaluation by others that impacts one's quality of life. Diagnoses of SAD become more common as children enter adolescence. Adolescents with Autism Spectrum Disorder (ASD) are diagnosed with comorbid SAD at higher rates than these individuals are diagnosed with other clinical disorders, including depression and other anxiety disorders. However, researchers do not yet know if teenagers with ASD and SAD demonstrate symptoms in their behavior and brains in the same way as teenagers with SAD and without ASD. One potential way to identify similarities and differences between these groups is through imaging the brains of teenagers with SAD without ASD, SAD and ASD, and teenagers with neither condition, while they learn fear associations. Fifty-seven adolescents participated in this study, with adolescents with ASD and SAD (n=17), SAD alone (n=20), and typically developing adolescents (n=20). All participants completed two fear conditioning and reversal studies while completing a functional magnetic resonance image (fMRI) scan of their brain. The study consisted of a Social condition (teenage faces paired with a scream sound) and Nonsocial condition (colored ovals paired with a loud white noise sound). Results revealed significant activation in a part of the brain associated with social information processing, the Inferior Temporal Gyrus (ITG), during fear conditioning. This activation was specifically seen within-groups, with the ASD with SAD group showing different activation of the ITG in the Social condition compared to the Nonsocial condition. The SAD group demonstrated differential activation between conditioning stimuli in the Nonsocial condition, but not in the Social condition. Results indicate that adolescents with ASD and SAD may display different neural mechanisms for acquiring fear compared to typically developing peers. Results have potential to inform treatment approaches.

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**Common and Distinct Mechanisms of Social Anxiety in Autism Spectrum Disorder**

Social Anxiety Disorder (SAD) is characterized by fear of negative evaluation during, or in anticipation of, social situations and avoidance of such situations (APA, 2013). SAD increases in prevalence as children enter adolescence, with a lifetime prevalence of up to 12.5 % (Kessler et al., 2005). Autism Spectrum Disorder (ASD) has an onset before 3 years of age (APA, 2013) and affects one in 59 individuals (Baio et al., 2018). Adolescents with ASD experience SAD at higher rates than other comorbidities, with up to 42-85 % of individuals with ASD also meeting criteria for SAD in their lifetime (de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007; Muris, Steerneman, Merckelbach, Holdrinet, & Meesters, 1998; Simonoff et al., 2008). The co-occurrence of these two disorders is more common than would be expected by chance, or base rates, alone. In addition to the higher than expected co-occurrence, there is evidence of a different developmental trajectory of SAD within ASD, such that adolescents with ASD report increased SAD symptoms with age, whereas children and adolescents without ASD report decreased behavioral avoidance (Kuusiko et al., 2008). Assessment of anxiety symptoms within individuals with ASD is complicated by behavioral features often observed within the disorder. For example, behavioral difficulties associated with ASD may be induced by anxiety (e.g., temper tantrums, social avoidance), and repetitive behaviors observed in ASD may be implemented as coping skills to decrease anxiety (e.g., body rocking, hand flapping; Wood & Gadow, 2010). Difficulty in ascertaining root causes of behavior make it potentially more difficult to treat anxiety symptoms in ASD.

Extant research has delineated several possible reasons for this increased comorbidity of ASD and SAD, such as impoverished social skills, emotion regulation difficulties, and difficulties with changing contingencies (executive function) observed in ASD. However, it is still not known whether the neural mechanisms underlying SAD within ASD are identical to SAD alone. This has implications for treatment. CBT-based interventions for anxiety in ASD and SAD are considered evidence-based interventions. However, effect sizes for response to intervention are currently smaller than what is observed in patients

with anxiety, but not ASD. Insight into different neural pathways may provide tools to improve, enhance, and individualize treatment for individuals with ASD and comorbid ASD. . One potential way to examine neural underpinnings of fear acquisition in ASD and SAD is to utilize a well-tested fear conditioning paradigm, which can quantify the brain regions underlying the acquisition of fear. A fear reversal paradigm can then be used to measure the modification of fear in the brain, which can inform how treatments for ASD and SAD as well as SAD alone may best work. Accordingly, the purpose of the proposed study is to characterize the neural and behavioral mechanisms and components of SAD in ASD and SAD as measured during fear conditioning and reversal in fMRI.

Common features and symptoms in ASD and SAD may contribute to high comorbidity rates of SAD in ASD. For instance, both conditions include deficits in social reciprocity, limited eye contact, social avoidance, and reduced verbal communication. Due to overlap in behaviors between individuals with ASD and SAD, it can be difficult for clinicians to assign a differential diagnosis between the two disorders (White, Schry, & Kreiser, 2014). Correct diagnosis of SAD in ASD is important, as treatment between the two disorders is distinct (e.g., exposure and cognitive behavioral therapy for SAD, versus social skills training in ASD).

Studies of comorbidity in ASD have consistently demonstrated clinically significant levels of anxiety within ASD (Siminoff et al., 2008; Lugnegård et al., 2011). SAD in particular is the most common comorbid anxiety condition in ASD (Kuusiko et al., 2008). Although a variety of explanations have been hypothesized to explain the high rate of comorbid SAD in ASD, one ecologically valid theory posits that core features of ASD may predispose these individuals towards anxiety (Wood & Gadow, 2010). For example, difficulty with social interactions, a core symptom of ASD (APA, 2013), may lead to social rejection among peers (Wood & Gadow, 2010). Social rejection may then lead to fear of performance in social situations, which then leads to behavioral and situational avoidance of social interactions, both of which are prominent feature of Social Anxiety Disorder (APA, 2013).

Despite high levels of anxiety in ASD, the overlap in symptoms of anxiety disorders and ASD complicates study of the phenomenology of anxiety symptoms in relationship to ASD. One potential limitation of characterization is the dearth of measurement tools for this population (Kerns et al, 2012; Kreiser et al., 2014). A review of measurement of anxiety in ASD revealed that anxiety has largely depended on self-report questionnaires, and less commonly on clinical interviews (Kreiser & White, 2014). Both questionnaires and interviews are reliant upon insight into one's own experiences, a mechanism that is hypothesized to be impaired in ASD (Frith & Happé, 1994). The use of parent-report measures for an internalizing disorder within ASD can be limited by parental insight into the true nature of symptom expression (Kreiser & White, 2014). For example, negative responses to changes in routine may be attributed to anxiety in one child, but core symptoms of ASD in another. Additionally, parent-report of comorbidities in ASD has been found to differ from child report (Mazefsky et al., 2011). Due to measurement limitations, which may conflate core symptoms of ASD with SAD, the mechanisms of anxiety in ASD are difficult to measure. As such, a more objective, biologically derived, measure of anxiety may serve to validate clinical interviews and self-report of symptomatology.

A potential way to investigate the mechanism of fear acquisition in individuals with ASD is the classic fear conditioning paradigm. Fear conditioning paradigms are simple and ecologically valid experimental models of anxiety disorders that have been found to reliably index adaptive and maladaptive fear learning, even across diverse study methods (Fullana et al., 2015). Fear conditioning paradigms via fMRI are uniquely poised to examine potential differences between clinical groups because they allow for spatial and temporal examination of these neural processes (Pine, 2009). Understanding how fears are acquired, on a neural level, can provide insight into ASD and SAD, including whether fear is learned via the same brain mechanisms. Traditional fear conditioning involves reliably pairing an intrinsically aversive stimulus, termed an unconditioned stimulus (US), with a neutral stimulus (CS+). The CS+ is compared to a neutral stimulus that has not been paired with an aversive stimulus (CS-). Pairing the neutral stimulus with the aversive stimulus results in new associative memory. After this learning has taken place,

new presentations of the neutral stimulus elicit anticipatory fear. Fear conditioning in humans can be observed through neuroimaging. Specifically, meta-analyses of neuroimaging studies of fear conditioning reveal a robust "fear network" within the brain, which include the dorsal anterior cingulate cortex (dACC), bilateral anterior cingulate cortex (AIC), amygdala, orbitofrontal cortex (OFC), anterior thalamus, ventral putamen (Etkin & Wager, 2005, Fullana et al., 2016). Examining this fear network across adolescents with ASD compared to those with SAD can elucidate whether neural underpinnings of fear learning are altered with ASD.

Examining neural responses serves as an additional unit of analysis above and beyond behavioral observations. Neuroimaging of fear may be particularly useful in adolescent clinical samples, such as ASD and SAD, given that youths may lack insight or language to describe their symptoms, or be otherwise unwilling and unable to disclose problems. Additionally, adolescents with SAD and ASD alike have demonstrated differences in either self-report or physiological differences compared to typically developing peers. For example, individuals with ASD were asked to observe pleasant, neutral, and aversive stimuli while skin conductance was collected (Shalom et al., 2006) In this study, the participants with ASD maintained similar levels of physiological arousal as their typically developing peers. However, individuals with ASD differed in their self-reports from controls, such that they reported the "unpleasant" images as being more pleasant and the "pleasant" images as more unpleasant than controls (Shalom et al., 2006). The authors of this study speculated that these discrepant findings were due to differences in cognitive emotional processing (medial prefrontal cortex) and physiological emotion processing (amygdala); that individuals with ASD may display near-typical amygdala responsiveness, but reduced self-awareness to interpret their own emotional states. Meanwhile, adolescents with SAD have also demonstrated the opposite pattern, of disparate biological responses despite similar self-report data relative to non-anxious peers (Britton et al., 2013). Specifically, adolescents with SAD evidenced reduced activation in the subgenual ACC compared to controls during neuroimaging while rating their fear

responses (Lau et al., 2013). Together, this indicates that biological markers of emotional responses may provide additional insight for clinical groups that is not observable in behavior alone.

The significance of the current project lies in the potential clinical implications for distinguishing specific mechanisms of fear acquisition in adolescents with ASD and comorbid anxiety. As previously stated, overlapping symptomatology increases difficulty with differential diagnosis for ASD and SAD. Improved measurement of the mechanisms of SAD in ASD is critical to begin effective treatment. Best practice treatments for SAD in ASD indicate that there is room to improve treatments. For instance, a recent meta-analysis determined that there was no significant difference in improvement between experimental and control conditions (SMD = 0.55, *p* values = 0.13) among treatment studies targeting comorbid anxiety disorders in youth with ASD (Kreslins, Robertson, & Melville, 2015). Although parents and clinicians observe statistically significant change in anxiety symptoms (Parents: SMD = 1.00; *p* = 0.01; clinician: SMD = 1.05, *p* = 0.0006; Kreslins et al., 2015), these changes do not reach clinical significance. Harnessing basic science techniques (i.e., fear conditioning and reversal paradigms) can inform intervention decisions based on neural mechanisms associated with acquiring fear may provide insight for whether exposure, cognitive, or third-wave mindfulness therapies may best dampen the fear responses. For example, reduced frontal lobe activation may indicate increases in cognitive-based (e.g., modification of thoughts or interpretation of events) may be needed to down-regulate emotional responses. Conversely, activation of both prefrontal and thalamic or amygdala functioning may indicate that exposure- and behavior-based therapies may be better suited to learn new associations. Finally, activation within self-referential brain regions, such as the precuneus, may indicate third-wave, mindfulness based interventions could be beneficial in order to retrain automatic biases.

Modifying a learned fear response has been extensively studied via fear extinction, and more recently through fear reversal paradigms. Both fear extinction and reversal hold potential for translating basic science research into treatments developed based on neural underpinnings of fear modification (Phelps, Delgado, Nearing, & LeDoux, 2004). Fear reversal requires a modulation of a fear that requires

learning a new association (Bouton, 2002) by utilizing prefrontal brain structures (Shechner et al., 2014). A typical fear reversal paradigm has a CS+ (paired with a US) and a CS- (not paired with a US). A reversal then has the CS+ no longer paired with the US, while the CS- is paired with the US (becoming a new CS+). The fear reversal paradigm enables study of the response when fear switches from one stimulus to another without developing generalized or perseverative fear (Schiller et al., 2008). Neuroimaging during fear reversal in clinical groups can be used to examine the neural mechanisms utilized to modify a fear response. Comparing the neural responses to a control group of nonanxious peers provides information regarding brain regions that are either over or underactive when modifying a fear response.

Work in adults with SAD has found prefrontal areas to mediate fear conditioning and reversal (Schiller et al., 2008). However, comparatively few neuroimaging studies have examined fear conditioning in adolescents with SAD. These studies suggest that adolescents with SAD rely more on subcortical regions, such as the amygdala, than prefrontal regions (Shechner et al., 2014; Lau et al., 2011). This indicates that the cognitive alteration of fear responses through prefrontal mechanisms is a process that occurs later in development. In a novel paradigm, which utilized human fear faces paired with a scream for the CS+ and collected self-reported anxiety, Lau and colleagues (2008) measured subjective anxiety in adolescents with SAD versus controls. In this study, adolescents with SAD rated greater fear of the CS+. Additionally, adolescents with anxiety have demonstrated reduced inhibition of the extinction response, or a heightened fear response, when returning for a second day of scanning (Pine, 2009). This is potentially indicative of reduced plasticity in the fear circuit in adolescents with anxiety. As such, a paradigm that more immediately indexes the flexibility required for successful fear extinction, such as a fear reversal task, can directly test plasticity of fear circuitry.

A relatively small number of studies have examined mechanisms of fear conditioning or reversal in ASD. Although fear conditioning and reversal in ASD has not been studied in individuals with a known co-morbid diagnosis, adults with ASD demonstrated deficits in learning that the previously “unsafe” cue is safe after fear reversal (South et al., 2011). Instead, individuals with ASD evidenced generalized fear

learning, or continuing to respond to the CS with fear despite the CS no longer being paired with a UCS. In this study, South and colleagues (2011) found a positive correlation between self-reported SAD and fear conditioning, such that greater amplitude of fear conditioning measured via skin conductance was associated with higher SAD symptoms according to self-report on the SCARED. In the only fMRI study published in ASD, adolescents with ASD displayed reduced responsiveness in the amygdala compared to controls, meaning that they responded to both the CS+ and CS- with greater amygdala activation (Top et al., 2016).

Fear conditioned responses are also associated with behavioral symptoms and subjective ratings of anxiety. For example, in adolescents and adults ASD, a study examining skin conductance as a proxy for strength of fear conditioning a relationship was observed between the conditioned response and social anxiety symptoms, with greater symptoms of SAD associated with a stronger fear response, or greater skin conductance (South et al., 2011). In a startle probe study of adults with SAD and controls, participants viewed social faces and received verbal feedback that was positive, negative, or neutral paired with faces that were happy, neutral, or sad (Lissek et al., 2008). In this study, participant's self-reported social anxiety symptoms were significantly correlated with the strength of their startle responses, such that higher SAD ratings were associated with increased startle reflex.

The purpose of the research proposed here is to characterize the neural response of fear acquisition and modification adolescents with ASD and SAD, with SAD alone, and with neither condition. Using a fear conditioning and reversal paradigm, fear acquisition and modification, or reversal, were measured by fMRI in a sample of adolescents with ASD and SAD (herein called the "ASD" group), SAD alone (herein called the "SAD" group) and typically developing controls (herein called the CON group). The central hypothesis was that groups would differ in neural bases of fear conditioning, such that amygdala activation for events and conditions would vary by group. Second, based on prior work demonstrating reduced amygdala responsivity in adolescents with ASD, it was hypothesized that that adolescents in the ASD would show reduced amygdala differentiation between stimuli compared to SAD and CON groups during

fear reversal during both social and nonsocial fear reversal. Third, based on research finding a generalized fear in ASD in reversal paradigms, it was hypothesized that the ASD group would continue to demonstrate fear to the CS+, even after the reversal condition cues this stimulus as safe, compared to the SAD and control group. Fourth, it was hypothesized that neural activation during fear conditioning and reversal would be associated with measures of anxiety.

## Methods

### Participants

Adolescents (N=57) between the ages of 12 and 17 were enrolled in this study for three groups ASD (n=17), SAD (n=20), and control (n=20). Demographic information for all participants is depicted in Table 1. Participants were recruited through mailing lists, flyers, and recruitment databases maintained through the department. Due to technical difficulties, some fMRI data were unable to be analyzed. Demographic information of participants that are included in fMRI analyses and those who are not is depicted in Table 2. Three participants with ASD who were eligible for the fMRI were unable to complete the scan (one due to extreme anxiety and comorbid selective mutism, two due to difficulty fitting in the scanner). One CON participant was unable to complete the fMRI due to claustrophobia once inside the scanner.

[TABLES 1 AND 2 GO HERE]

#### Inclusion criteria

Participants were included in the study if they met the following criteria: aged 12-17, FSIQ of 80 or above, normal or corrected to normal vision, capable of making an informed decision. The ASD and Social Anxiety groups must have a diagnosis of SA as determined by a CSR of 4 or higher on the ADIS-5 Social Anxiety Disorder module by *either* the child or the parent (SA and ASD and Social Anxiety group). The ASD group had an ADOS-2 score above clinical cut-off.

#### Exclusion criteria

MRI contra-indications (e.g., metal in body, braces).

### Procedures

Interested participants completed an eligibility phone screen, typically completed by the parent. Contingent on meeting exclusion criteria, participants completed two visits for the study. The first visit was an additional eligibility screening, confirming diagnostic status. During this visit, participants also completed the measures described in detail below. The final visit consisted of the fMRI task, described in detail below.

## Measures

### Autism Diagnostic Observation Schedule – 2 (ADOS-2)

The ADOS-2 (Rutter et al., 2012) is a semi-structured interview and is considered part of the clinical "gold standard" for assessment of the presence or absence of ASD. Modules were administered based on developmental level, with Module 3 (Fluent Speech) and Module 4 (Adolescent/Adult) modules administered. Symptoms of ASD were rates 0-3 by the examiner, with algorithm items transferred providing clinical cut-offs for significant symptoms of autism. The ADOS has demonstrated sensitivity in the upper 90% range and specificity in the upper 80% to lower 90% range (Lord et al., 2008). For the four relevant modules, inter-rater agreement on diagnostic conclusions ranged from 0.81 to 0.93. The internal consistency for all domains and modules ranges from 0.47 to 0.94. This measure was only administered to the ASD group to confirm diagnosis, and only adolescents above clinical cut-off were included in the fMRI sample.

### The Weschler Abbreviated Scales of Intelligence - II (2 subtests; WASI-2)

The WASI-2 edition (Wechsler, 1999) provides a brief and reliable measure of intelligence. For the present study, the full scale IQ score derived from two subtests was used. The first of these is the vocabulary subtest which pertains to verbal comprehension. Briefly, the test involves the examinee defining a series of words that are presented visually and orally. During the matrix reasoning subtest, which taps into fluid and broad visual or spatial intelligence, the administrator shows the examinee an

incomplete matrix or series and the examinee selects the response that completes it. Psychometric figures for the WASI-II are available for child and adult samples. Reliability for the composites are strong for all the included modules: verbal comprehension (child: $r = 0.91$), matrix reasoning (child: $r = 0.87$)), and the Full Scale IQ-2 (child: $r = 0.93$). The WASI-II was collected to measure potential differences between groups in general intelligence.

**Social Responsiveness Scale-2 (SRS-2)**

The SRS-2 (Constantino, 2003) is a well-validated measure of social impairment across settings. This measure detects subtle differences between groups. The SRS-2 consists of 65 items scored 1-4 (1= not at all true, 2 = sometimes true, 3 = often true, 4 = almost always true). Scores are converted to subscales, Awareness, cognition, communication, motor, and restricted and repetitive behaviors, as well as the total score. Raw scores are converted to T scores, which were used in analyses. The SRS has demonstrated strong internal consistency ( $a = 0.93$-$0.97$), and acceptable long-term test construct delay stability ($r = 0.77$-$0.85$). Further, inter-rater agreement between parents ($r = 0.91$), and between parent-teacher ($r = 0.75$-$0.82$) was also strong. As far as the subscales go, all of them showed strong to very strong internal consistency, ranging from 0.77 to 0.92. This measure was collected to determine whether severity of autism symptoms were correlated with fear learning and modification.

**Anxiety Disorders Interview Schedule - 5**

The ADIS-5 (Albano, Chorpita, & Barlow, 2003) is a semi-structured clinical interview designed to assign DSM-5 diagnosis. For the purposes of this project, the graduate clinician only administered the Social Anxiety module. This assessment was conducted with both the adolescent and parent. Clinical severity was reported by the clinician on a 9 point scale (0-8), where 0 = not at all, 4 = clinically significant, and 8 = very very high symptoms. The most recent study addressing independent administrations of the previous ADIS-IV found inter-rater agreement to range from good to excellent ( $k = 0.67$-$0.86$; Ashbaugh,

2015). Composite scores were calculated, which consisted of the highest clinical severity rating form either the parent or the child.

**Youth Self Report (YSR)**

The YSR (Achenbach & Ruffle, 2000) self-report form measures symptom severity across a wide range clinical disorders. Adolescents answered how true various statements about the emotional functioning are for them on a three point scale (0=Not true, 1= Somewhat True, 2 = Very True). This measure produces T scores for six DSM-oriented scales: affective problems, anxiety problems, attention/deficit/hyperactivity problems, conduct problems, oppositional defiant problems, and somatic problems. Reliability coefficients (Cronbach's alpha coefficients) are favorable, ranging from .71 to .89 (Nakamura et al., 2009). The purpose of this measure was to examine child ratings of severity of anxiety.

**Child Behavior Checklist (CBCL)**

The CBCL (Achenbach & Ruffle, 2000) parent-report form measures symptom severity across wide range clinical disorders. Parents answered how true various statements about the emotional functioning are for their child on a three point scale (0=Not true, 1= Somewhat True, 2 = Very True). This measure produces T scores for six DSM-oriented scales: affective problems, anxiety problems, attention/deficit/hyperactivity problems, conduct problems, oppositional defiant problems, and somatic problems. Reliability coefficients (Cronbach's alpha coefficients) are favorable, ranging from .71 to .89 (Nakamura et al., 2009). The purpose of this measure was to examine parental ratings of severity of anxiety.

**Liebowitz Social Anxiety Scale for Children and Adolescents (LSAS-CA)**

The LSAS-CA (Masia-Warner et al., 2003) is a well-validated self-report measure of social anxiety along two subscales, fear and avoidance, as well as a total score. This measure has high internal consistency (alpha = .90-.97) and relates more closely to social anxiety. Adolescents answered questions

about how afraid, and how much they avoid different situations on a four point scale (0=None, 1=mild, 2=moderate, 3=severe). All adolescents completed this form. This questionnaire was used as a correlate of self-perceived social anxiety.

**Pubertal Development Scale (PDS)**

The PDS (Carskadon & Acebo, 1993) is a self-reported measure of pubertal status. Adolescents completed this measure at their scanning appointment, to ensure the time between this measure and the scan was matched. All teens answered questions about height, body hair, and skin changes. Males and females answered supplemental questions about voice changes and menarche respectively. Questions were completed on a 4 point scale (1= has not started, 2= has barely started, 3=is definitely underway, 4 = seems completed) with the exception of menarche, which was reported on a two point scale (present or not). This scale is comparable to Tanner stages of development, but is less invasive (Caskadon & Acebo, 1993). This scale was administered to co-vary results according to pubertal maturation, which has known neural effects.

**MRI Acquisition**

MRI data acquisition was approximately 30 minutes in duration (5 minutes T1-weighted structural, 25 minutes for the Fear Conditioning and Reversal conditions). All MRI scanning was performed on a Siemens 3T Tim Trio system outfitted with a 45 mT/m gradient system (200 T/m/s slew rate), 8 receive channels, and a 12 channel head coil. T1 weighted anatomical images were acquired with a 3D MPRAGE sequence at 1 mm isotropic resolution and scanning parameters: TI = 900 ms, TR = 2.6 s, TE = 3 ms, FA = 8°, BW = 130 Hz/pixel, and GRAPPA parallel imaging factor of 2, for an acquisition time of 4 minutes and 38 seconds. fMRI data was acquired using a T2* weighted single-shot EPI sequence. Whole-brain functional volumes were acquired in 30 3.4-mm axial slices (with a 20% slice gap), each with 3.44 mm x 3.44 mm in-plane resolution. Additional fMRI imaging parameters include: TR = 2 s, TE = 30 ms, FA = 90°, BW = 2442 Hz/pixel.

**fMRI task**

The fMRI task consisted of a two by two design, with social and nonsocial conditions, with both conditions engaging in a fear conditioning and reversal phase (Fig. 1). Conditions were counterbalanced within groups, with four different randomization options, "A", "B", "C", and "D." The first image (face or oval) shown in each run was the "CSPlus" event during the acquisition phase, and the "CSMinus-Reversed" event during the reversal phase. The second face or oval shown is the "CSMinus" during the acquisition phase and "CSPlus-Reversed" during the reversal phase. Block A consisted of the social condition with the Caucasian adolescent first, and the blue oval first, block B consisted of the nonsocial condition first, with the blue oval first, and for the social condition the Caucasian adolescent face first. Block C consisted of the social condition first with the African American adolescent first and the yellow oval first for the nonsocial condition. In block D, the nonsocial condition was shown first, with the yellow was first, in the nonsocial run the African American adolescent was shown first. The paradigm consisted of a baseline phase, with 12 trials lasting 8 seconds each. Consistent with Schiller et al. (2008) fear reversal paradigm, the acquisition phase consisted of 12 presentations of the CS paired with the UCS. The reversal phase was not signaled, and consisted of 12 presentations of the CS with the reversed UCS. Presentations of events were pseudorandomized, to maximize the neural signal. The social fear conditioning task was adapted from the paradigm used by Lau et al. (2008) due to the ease of use with adolescent participants, particularly those with SA.

[ FIGURE 1 GOES HERE]

During the fMRI task, participants were asked to make four ratings for each stimuli: 1) how much do they like the image, 2) how pleasant is the image, 3) how unpleasant is the image, and 4) how afraid of the image are they. Participants were prompted to provide these ratings at five time points, 1) before

the experiment began, 2) early in the acquisition phase, 3) late in the acquisition phase, 4) early in the reversal phase, and 5) late in the reversal phase. Participants were asked to provide their ratings on a scale of 1-9, where 1 was "not at all" and 9 was "very very much."

**fMRI Data processing**

Slice time correction, motion correction, and spatial smoothing (Gaussian kernel with full-width-at-half-maximum of 2.0 × the voxel size) were performed on the data. Similar to the analyses conducted by Britton et al. (2013), I restricted analyses by creating regional masks for the amygdala, insula, vmPFC, and dlPFC to determine cluster thresholds.

Statistical models were set for the onset of each event type, including Acquisition (CSMinus, CSPlus) as well as for reversal (CSMinus-Reversed, CSPlus-Reversed) and to create subject-level Beta-coefficients for the regions of interest indicated above. Beta-coefficients were fit into an ANOVA to test for a group difference (ASD, SAD, CON) for fear reversal and conditioning. False discovery adjustments were implemented to account for multiplicity when performing comparisons for fMRI data. GLM-based analysis were run in FSL. To examine significant F-test results, follow-up *t*-tests comparing ASD to SAD and CON within brain regions of interest (dlPFC and amygdala) were performed.

**Data analysis**

**Behavioral Analyses.** To determine whether groups differed on self-, parent-, and clinician-report measures, ANOVAs were conducted to determine mean differences on behavioral measures of anxiety. Post-hoc independent samples *t*-tests to measure whether groups differ on behavioral measures of anxiety, in terms of self-report, clinician-assessment, and parent report.

**Hypothesis testing.** To test the first hypothesis, that groups would differ in fear conditioning, such that amygdala activation for events and conditions would vary by group, 3 Group (ASD, SAD, CON) X 2 Event (CSPlus, CSMinus) X 2 Condition (social, nonsocial) ANOVAs were conducted. First, a whole-

brain analysis was conducted. Then, the same ANOVA constricted by brain regions of interest, the amygdala and dlPFC, was conducted.

To test the second hypothesis, that the ASD group would demonstrate reduced amygdala responsivity in the reversal condition compared to the SAD and CON groups, a 3 Group (ASD, SAD, CON) X 2 Event (CSPlus-Reversed, CSMinus-Reversed) X 2 Condition (social, nonsocial) ANOVA was conducted. The ANOVA was conducted first as a whole-brain analysis, and second with regions of interest constricted to the amygdala.

To test the third hypothesis, that the ASD group would demonstrate generalized fear in the reversal phase, two 3 X 2 ANOVAs were conducted, consisting of Group (ASD, SAD, CON) X 2 Event (CSPlus, CSPlus-Reversed), and 3 Group (ASD, SAD, CON) X Event (CSMinus, CSMinus-Reversed). It was hypothesized that the ASD group would continue to demonstrate amygdala activation during the CSMinus-Reversed condition compared to the SAD and CON group,

To test the fourth hypothesis that significant neural activation observed during conditioning would be associated with behavioral indicators of anxiety, any significant activation observed across conditions was correlated with anxiety measures (LSAS, ADIS, CBCL, YSR).

## Results

### Behavioral Analyses

To examine whether groups differed in self-, parent-, and interview-ratings of participant anxiety, a series of one-way, three-group ANOVAs were conducted. Results indicated that groups differed significantly on measures of anxiety. On the LSAS-CA, there were significant differences on the Total Score ($F(2, 57) = 9.321$, $p = 0.0003$). A post hoc Tukey test showed that the SAD group differed from the TD group significantly ($p = 0.0002$), such that the SAD group reported greater symptoms of social anxiety. There was no significant difference between the SAD and ASD group ($p = 0.053$), although the SAD group reported more symptoms than the ASD group. The ASD group and TD group did not differ from each other ($p = 0.19$). When examining the subscales of the LSAS, there was a significant difference on the Fear subscale ($F(2, 57) = 13.28$, $p > 0.0001$). A post hoc Tukey test demonstrated significant differences between all groups (all $ps > 0.05$), such that the SAD group reported greater symptoms than the ASD and TD groups, and the ASD group reported greater symptoms than the TD group. On the Avoidance subtest of the LSAS-CA there was a significant difference ($F(2, 57) = 5.629$, $p = 0.006$). Post hoc Tukey tests showed that the SAD group was significantly different from the TD group ($p = 0.004$), reporting greater symptoms of social anxiety. The ASD group did not significantly different from the TD or SAD group (all $ps < 0.05$). LSAS scores are depicted in Figure 2.

[FIGURE 2 GOES HERE]

On the ADIS, ANOVAs were conducted for parent and child report of interference, as well as clinical severity rating (CSR). Parent reports of interference were significantly different ($F(2, 57) = 73.85$, $p > 0.0001$). Post hoc Tukey tests revealed that each group was significantly different from each

other (all $p$'s < 0.001), with the ASD group demonstrating the greatest parent reported interference for SAD symptoms, followed by the SAD group and then the TD group. Clinician reported CSR scores were significantly different ($F(2, 57) = 85.08$, $p > 0.0001$). Both the ASD and SAD group significantly differed from the TD group ($ps < 0.0001$), but did not differ from each other ($p = 0.19$). For child reported interference, there was a significant difference ($F(2, 57) = 7.466$, $p = 0.0001$). Post hoc Tukey tests revealed that the SAD group and TD group were significantly different ($p = 0.0009$), with the SAD group endorsing greater interference. The ASD group did not differ significantly from the TD group or the SAD group. Clinician rated CSR scores revealed a significant difference ($F(2, 57) = 28.47$, $p = 0.0001$). Post hoc Tukey tests indicated that there was a significant difference between all groups, with the SAD group significantly elevated compared to the ASD group ($p = 0.02$) and TD group ($p < 0.0001$). The ASD group had significantly greater impairment than the TD group ($p = 0.0001$). ADIS scores are shown in Figure 3.

[FIGURE 3 GOES HERE]

On the ASEBA measures, ANOVAs were conducted for parent and child report of anxiety symptoms. T-scores for anxiety on the CBCL were significantly different ($F(2,54) = 18.79$, $p = 0.0001$). Post hoc Tukey tests indicated that the TD group was rated as having significantly less parent-reported anxiety than both the ASD group ($p > 0.0001$) and SAD group ($p > 0.0001$), but that the two clinical groups were not significantly different from each other ($p = 0.84$). T-scores for the YSR were significantly different ($F(2,53) = 9.2$, $p = 0.0003$). Post hoc Tukey tests indicated that the SAD group reported significantly greater anxiety than the CON group ($p = 0.002$), and that the ASD group reported significantly greater symptoms of anxiety than the CON group ($p = 0.002$). The SAD and ASD groups did not differ from each other ($p = 0.97$).

[TABLE 3 GOES HERE]

**Primary results**

To test the first hypothesis, that group differences in are present in fear conditioning for social compared to nonsocial stimuli, ANOVAs were conducted across the whole brain. For the primary contrast of interest, a 2 (Event) X 2 (Condition) X 3 (Group) ANOVA whether Groups differed in whole brain activation in Event type (CSPlus, CSMinus) by Condition (Social, Nonsocial) activation, there was significant activation in the Inferior Temporal Gyrus (ITG; depicted in Figure 4) and Lateral Occipital Cortex (Figure 5) were observed ($p < 0.001$). To examine this interaction further, a Region of Interest (ROI) analysis was conducted, with mean values of activation extracted from the ITG for Event and Condition. Results indicated the Main effect of Event ($F(1,40) = 7.601$, $p = .009$) and Condition ($F(1,40) = 8.870$, $p = 0.005$) were driving the significant activation observed, but not for Group. No significant effects for Group X Condition or Group X Event were observed. Results for the ROI analysis are depicted in Figure 5. These results indicates that the hypothesis was partially supported, such that event type and condition modulated neural responses, but that conditioning did not vary as a function of group, and the amygdala and prefrontal cortex were not the primary regions of activation.

[FIGURES 4 AND 5 GO HERE]

To further probe the effects within this ROI, follow-up *t*-tests were conducted for the Nonsocial CSPlus and CSMinus ($t(1,40) = -3.008$, $p = .005$), such that there was greater activation during the CSMinus condition compared to the CSPlus condition. The Social CSPlus contrast was also significantly different from the Nonsocial CSPlus ($t(1,40) = 2.095$, $p = 0.045$), such that the Social CSPlus had significantly greater activation than the Nonsocial CSPlus. The contrasts between Social CSPlus and Social CSMinus, and the Social and Nonsocial CSMinus were not significantly different from each other.

To examine within-group effects, *t*-tests were conducted. In the ASD group, a significant effect was observed in the Social CSPlus compared to the Nonsocial CSPlus condition ($t(,1,8) = 1.23$, $p = 0.033$), such that the Social CSPlus condition had greater activation than the Nonsocial CSPlus condition. No other significant comparisons were observed in the ASD group. In the SAD group, a significant difference was observed in the Nonsocial CSPlus compared to the Nonsocial CSMinus ($t(1,17) = -2.69$, $p = 0.017$), such that the Nonsocial CSMinus had greater activation than the Nonsocial CSPlus. No other significant differences were observed in the SAD group. No significant within-group differences were observed in the CON group.

[FIGURE 6 GOES HERE]

To examine simple effects for the Acquisition phase, a of 2 (Condition) X 3 (Group) ANOVA were conducted for the CSPlus. A Main effect in the ASD group was observed, such that participants with ASD had greater activation in the Lateral Occipital Cortex. There was also a Main effect in the SAD group, such that participants with SAD evidenced greater activation in the Lateral Occipital Cortex and precuneus. These results indicate that participants with ASD and SAD processed Social fear conditioning in these respective regions more than Nonsocial fear conditioning. There was no Main effect for the CON group, and no Interaction was observed. This indicates that the CON group did not engage brain regions significantly differently for processing the Social fear conditioning compared to the Nonsocial fear conditioning. When comparing Nonsocial activation as greater than Social activation, no significant differences were observed. No significant effects were observed elsewhere in the brain for Condition for the CSPlus. Collectively, these results partially supported the primary hypothesis, indicating that the SAD and ASD demonstrated differential activation for condition, although the amygdala and prefrontal cortex were not the primary regions of activation.

For the simple effect of the CSMinus condition, where Social activation was greater than Nonsocial activation, no significant differences were observed. This indicates that no group processed Social CSMinus more in any brain region than the Nonsocial CSMinus. When Nonsocial activation was greater than Social activation, there was a Main effect in the CON group, such that CON participants had greater activation in the Lateral Occipital Cortex. There was also a Main effect in the SAD group, such that participants with SAD evidenced greater activation in the Lateral Occipital Cortex. There was no main effect for the ASD group, and no interaction was observed. These results indicate that the lateral occipital cortex was used to process nonsocial compared to social information more for CON and SAD participants. The ASD group did not demonstrate significant activation between conditions in the lateral occipital cortex. This indicates that differences in social and nonsocial processing in the lateral occipital cortex are due to reduced activation in the ASD group during the CSMinus condition. Results partially support the primary hypothesis, such that SAD and CON groups demonstrated differential activation across conditions, however, the hypothesized regions of amygdala and prefrontal cortex were not differentially active.

To test the second hypothesis, that the ASD group would demonstrate differential amygdala activation during the Reversal conditions, ANOVAs were conducted. The primary 3 (Group) X 2 (Condition) X 2 (Event) ANOVA examined the difference of whether the CSPlus-Reversed is significantly different than the CSMinus-Reversed condition. No significant differences were observed for when the Social condition was greater than the Nonsocial condition, or when the Nonsocial condition was greater than the Social condition. This finding does not support the second hypothesis, that adolescents with ASD would demonstrated reduced amygdala differentiation during reversal compared to the SAD and CON groups.

To further test the second hypothesis, 3 (Group) X 2 (Condition) analyses were conducted for the CSPlus-Reversed and CS-Minus-Reversed. A significant Main effect observed for the SAD group when the Social reversal was greater than the Nonsocial reversal in the Middle Frontal Gyrus. No significant

activation was observed during the CSMinus-Reversed condition. Although this result does not support the second hypothesis, that the ASD group would show differential activation of the amygdala during fear reversal, it does demonstrate additional resources utilized in the SAD group during social reversal conditions.

To test the third hypothesis, that the ASD group would demonstrate perseverative fear responses, 3 (Group) x 2 (Condition) X 2 (Event) ANOVAs were conducted, where events were the acquisition and reversal phase. When comparing CSPlus to the CSPlus-Reversed no significant effects were observed. Similarly, no significant effects were observed for the CSMinus compared to the CSMinus-Reversed. This indicates that there was no significant difference in how the groups processed initial fear conditioning to the fear reversal conditions, either within- or between-groups. This result does not support the third hypothesis, that participants with ASD demonstrate perseverative fear. Activation table depicting significant activation clusters and regions is presented in Table 4.

[TABLE 4 GOES HERE]

**Brain Behavior Relationships**

To test the fourth hypothesis, that neural activation during fear conditioning was associated with measures of anxiety, correlations were conducted between ITG activation and self-, parent- and clinician-rated anxiety in the participants. First, correlations were conducted between the acquisition CSPlus events for condition (Social and Nonsocial) correlated with LSAS scores (Fear, Avoidance, and Total, ADIS (parent-and child-rated CSR, clinician interference ratings) and ASEBA measures (YSR and CBCL Anxiety). No significant correlations were observed between anxiety measures and CSPlus. Second, correlations were conducted for the CSMinus events for condition (Social and Nonsocial). After testing for outliers, a significant correlation was observed between parent-rated interference of SAD symptoms

on the ADIS and the nonsocial condition CSPlus ($p$ = 0.04, $r$= -0.33), such that as interference of SAD increased, ITG activation decreased. This finding does not support the hypothesis that increased activation during conditioning would be associated with greater anxiety symptoms (depicted in Figure 7).

[FIGURE 7 GOES HERE]

**fMRI Behavioral Analyses**

To examine whether groups differed in self-reported ratings during the fMRI for how much participants a) liked the image, found the image b) pleasant, c) unpleasant, or were d) afraid of the image, ANOVAs were conducted. First, differences in responses for the social stimuli were conducted. For how well participants liked the first face, no significant group differences were observed for the Baseline condition ($F$(2,52) = 0.015, $p$ = 0.99), first Acquisition condition ($F$(2,52) = 0.326, $p$ = 0.72), second Acquisition condition ($F$(2,52) = 0.424, $p$ = 0.66), first Reversal condition ($F$(2,52) = 0.814, $p$ = 0.45), or second Reversal condition($F$(2,52) = 0.059, $p$ = 0.94). For how well participants liked the second face, there were no significant group differences were observed for the Baseline condition ($F$(2,52) = 0.169, $p$ = 0.85), first Acquisition condition ($F$(2,52) = 0.027, $p$ = 0.97), second Acquisition condition ($F$(2,52) = 0.177, $p$ = 0.83), first Reversal condition ($F$(2,52) = 0.444, $p$ = 0.64), or second Reversal condition($F$(2,52) = 0.294, $p$ = 0.75). Ratings for the Social Like condition are depicted in Figure 8.

[FIGURE 8 GOES HERE]

For how pleasant participants found the first face, no significant group differences were observed for the Baseline condition ($F$(2,52) = 0.422, $p$ = 0.66), first Acquisition condition ($F$(2,52) = 0.461, $p$ = 0.63), second Acquisition condition ($F$(2,52) = 0.202, $p$ = 0.82), first Reversal condition ($F$(2,52) = 1.005,

$p = 0.37$), or second Reversal condition($F(2,52) = 1.399$, $p = 0.26$). For how pleasant participants found the second face, again there were no significant group differences were observed for the Baseline condition ($F(2,52) = 0.11$, $p = 0.90$), first Acquisition condition ($F(2,52) = 0.849$, $p = 0.43$), second Acquisition condition ($F(2,52) = 1.66$, $p = 0.55$), first Reversal condition ($F(2,52) = 0.602$, $p = 0.55$), or second Reversal condition($F(2,52) = 1.399$, $p = 0.26$). Ratings for the Social Pleasant condition are depicted in Figure 9.

[FIGURE 9 GOES HERE]

For how unpleasant participants found the first face, there were no significant group differences for the Baseline condition ($F(2,52) = 0.511$, $p = 0.603$), first Acquisition condition ($F(2,52) = 0.504$, $p = 0.61$), second Acquisition condition ($F(2,52) = 0.811$, $p = 0.45$), first Reversal condition ($F(2,52) = 1.656$, $p = 0.20$), or second Reversal condition($F(2,52) = 0.245$, $p = 0.78$). For how unpleasant participants found the second face, there was a significant difference for the Baseline condition ($F(2,52) = 3.208$, $p = 0.049$), such that the ASD group found the second face significantly more unpleasant than the SAD group ($p = 0.049$). There were no other significant differences for the first Acquisition condition ($F(2,52) = 0.876$, $p = 0.42$), second Acquisition condition ($F(2,52) = 0.388$, $p = 0.68$), first Reversal condition ($F(2,52) = 0.5$, $p = 0.59$), or second Reversal condition($F(2,52) = 1.282$, $p = 0.28$). These results are depicted in Figure 10.

[FIGURE 10 GOES HERE]

For how afraid participants were of the first face, there were no significant group differences for the Baseline condition ($F(2,52) = 0.014$, $p = 0.99$), first Acquisition condition ($F(2,52) = 0.098$, $p = 0.46$), second Acquisition condition ($F(2,52) = 0.532$, $p = 0.591$), first Reversal condition ($F(2,52) = 0.81$, $p =$

0.45), or second Reversal condition($F(2,52) = 0.908$, $p = 0.41$). For how afraid participants were of the second face, there were no significant differences for the Baseline condition ($F(2,52) = 0.656$, $p = 0.52$), the first Acquisition condition ($F(2,52) = 0.182$, $p = 0.83$), second Acquisition condition ($F(2,52) = 0.315$, $p = 0.73$), first Reversal condition ($F(2,52) = 0.941$, $p = 0.40$), or second Reversal condition($F(2,52) = 1.702$, $p = 0.19$). These results are depicted in Figure 11.

[FIGURE 11 GOES HERE]

For the Nonsocial condition, For how well participants liked the first oval, no significant group differences were observed for the Baseline condition ($F(2,52) = 0.305$, $p = 0.73$), first Acquisition condition ($F(2,52) = 0.197$, $p = 0.82$), second Acquisition condition ($F(2,52) = 0.158$, $p = 0.85$), first Reversal condition ($F(2,52) = 0.164$, $p = 0.85$), or second Reversal condition($F(2,52) = 0.287$, $p = 0.75$). For how well participants liked the second oval, there were no significant group differences were observed for the Baseline condition ($F(2,52) = 0.861$, $p = 0.43$), first Acquisition condition ($F(2,52) = 0.334$, $p = 0.72$), second Acquisition condition ($F(2,52) = 0.185$, $p = 0.83$), first Reversal condition ($F(2,52) = 0.255$, $p = 0.78$), or second Reversal condition($F(2,51) = 0.531$, $p = 0.59$). These results are depicted in Figure 12.

[FIGURE 12 GOES HERE]

For how pleasant participants found the first oval, there was a significant group differences for the Baseline condition ($F(2,52) = 3.56$, $p = 0.036$), such that the ASD group reported finding the first oval as significantly more pleasant than CON group ($p = 0.03$). There were no significant differences between groups for the first Acquisition condition ($F(2,52) = 0.023$, $p = 0.98$), second Acquisition condition ($F(2,52) = 0.606$, $p = 0.55$), first Reversal condition ($F(2,52) = 0.369$, $p = 0.69$), or second Reversal

condition($F(2,52) = 0.067$, $p = 0.94$). For how pleasant participants found the second oval, there were no significant differences for the Baseline condition ($F(2,52) = 1.53$, $p = 0.36$). There were no significant differences for the first Acquisition condition ($F(2,52) = 0.086$, $p = 0.92$), second Acquisition condition ($F(2,52) = 0.031$, $p = 0.97$), first Reversal condition ($F(2,52) = 0.019$, $p = 0.98$), or second Reversal condition($F(2,51) = 0.447$, $p = 0.64$). These results are depicted in Figure 13.

[FIGURE 13 GOES HERE]

For how unpleasant participants found the first oval, there were no significant group differences were observed for the Baseline condition ($F(2,52) = 0.342$, $p = 0.71$), first Acquisition condition ($F(2,52) = 0.965$, $p = 0.38$), second Acquisition condition ($F(2,52) = 0.516$, $p = 0.6$), first Reversal condition ($F(2,52) = 1.785$, $p = 0.178$), or second Reversal condition($F(2,51) = 0.083$, $p = 0.92$). For how unpleasant participants found the second oval, again there were no significant group differences were observed for the Baseline condition ($F(2,52) = 0.558$, $p = 0.58$), first Acquisition condition ($F(2,52) = 0.814$, $p = 0.45$), second Acquisition condition ($F(2,52) = 0.526$, $p = 0.59$), first Reversal condition ($F(2,52) = 0.129$, $p = 0.88$), or second Reversal condition($F(2,51) = 0.062$, $p = 0.94$). These results are depicted in Figure 14.

[FIGURE 14 GOES HERE]

For how afraid participants were of the first oval, there were no significant group differences for the Baseline condition ($F(2,52) = 0.17$, $p = 0.84$), first Acquisition condition ($F(2,52) = 0.16$, $p = 0.85$), second Acquisition condition ($F(2,52) = 0.341$, $p = 0.73$), first Reversal condition ($F(2,52) = 1.098$, $p = 0.34$), or second Reversal condition ($F(2,51) = 0.703$, $p = 0.5$). For how afraid participants were of the second oval, there were no significant differences for the Baseline condition ($F(2,52) = 0.898$, $p = 0.41$), the first Acquisition condition ($F(2,52) = 1.34$, $p = 0.27$), second Acquisition condition ($F(2,52) = 1.32$, $p$

= 0.28), first Reversal condition ($F(2,52) = 1.404$, $p = 0.26$), or second Reversal condition($F(2,51) = 0.073$, $p = 0.93$). These results are depicted in Figure 15.

[FIGURE 15 GOES HERE]

## Discussion

This is the first study to examine neural responses to social fear conditioning compared to nonsocial fear conditioning in participants with ASD and comorbid SAD. Despite the fact that research and clinical observations have indicated that individuals with ASD display heightened fear responses in social situations, previous research in fear conditioning and reversal in ASD has only utilized nonsocial stimuli (Bernier, Dawson, Panagiotides & Webb, 2005; South et al., 2011; Top et al., 2016;). Results of this study suggest differential fear conditioning among youth with ASD, those with SAD, and health controls. The current study found significant differences in neural responses to the CSPlus compared to the CSMinus for social compared to nonsocial conditions. Specifically, the ITG was differentially responsive based on both condition and event type, such that the ASD group exhibited differential activation in the Social condition for the CSPlus compared to the CSMinus, and the SAD group demonstrated differential activation in the ITG in the nonsocial condition for the CSPlus compared to the CSMinus. Accordingly, the ITG appears to differentiate anxiety to social compared to nonsocial stimuli in individuals with ASD. Notably, the ITG potentially served a different function in SAD alone, responding less to nonsocial CSPlus compared to CSMinus.

Extant research on fear conditioning in ASD has revealed conflicting results, with some studies indicating atypical fear conditioning in ASD (Gaigg et al., 2007; Top et al., 2015), while another study indicated that individuals with ASD display typical fear conditioning responses compared to controls (Bernier et al., 2005). The findings of the current study may potentially shed light on one potential mechanism observed within ASD that is differentially responsive to social fear compared to nonsocial fear, the ITG. The ASD group evidenced much greater activation of the ITG in the social condition. Activation of the temporal gyrus during emotional viewing tasks is associated with fear relevance (Sabatinelli, Bradley, Fitzsimmons & Lang, 2005). This indicates that the ASD group in particular found the social fear stimuli to be especially salient. Although neural patterns of activation of the ITG between the ASD and CON group were similar in the nonsocial condition, the ASD group demonstrated

enhanced activation in the ITG to social compared to nonsocial fear learning. This suggests in ASD uniquely, the ITG is differentially responsive to social fear stimuli, which is potentially a marker of an atypical processing system.

The temporal gyrus is associated with social information processing. Pelphrey and colleagues (2005) found socially-relevant activation in the temporal gyrus during an fMRI task, such that typically-developing adults who viewed social stimuli (eye, hand, mouth movement) demonstrated significant activation in the temporal gyrus. In ASD, the temporal gyrus has consistently been hypothesized to relate to dysfunction in social information processing, including eye gaze, social attention, and language learning (Redcay, 2008). Given the increased ITG activation observed in the ASD group to the social fear conditioning stimuli, the ASD group in particular is likely at greater risk for dysfunction in this brain region. Interestingly, the SAD and ASD groups evidenced relative deactivation of the ITG during the nonsocial fear acquisition condition. The relative deactivation during the nonsocial condition could indicate suppression of the ITG during fear conditioning that is nonsocial in nature. Together, these results indicate that the ITG may differentiate both between social and nonsocial stimuli, as well as between clinical groups.

In the present study, it was hypothesized that the ASD group would demonstrate reduced sensitivity in the amygdala to fear reversal. This was based on prior work on fear conditioning in adults with ASD, which indicated that the amygdala was hypoactive during fear extinction (Top et al., 2016). A review of neuroimaging research has demonstrated reduced activation of the limbic system, which is associated with the regulation of fear, especially when viewing social stimuli (Dichter, 2012). However, no significant differences in activation in the amygdala in the ASD group compared to either the SAD group or the CON group during the reversal condition were observed in the present study. This indicates that prior work on reduced amygdala sensitivity in ASD may be explained by other factors. For example, one possible reason for the discrepant findings between the current study and past literature may be attributable to sample selection. The current study selected for participants with comorbid SAD,

and screened out participants who did not meet criteria for SAD. In studies of adults with SAD, increased amygdala activation is reported in the literature (Birbaumer, 1998). Therefore, it is possible that the added effect of having both ASD and SAD resulted in a normalization of amygdala responsiveness in the ASD group. Alternatively, it is possible that the neural circuitry of fear conditioning develops through different mechanisms in teens with ASD, such as through the ITG, rather than through traditional neural circuitry.

It was additionally hypothesized that the ASD group would demonstrate perseverative, or generalized fear. Prior work has shown that participants with ASD are have difficulty learning that the previously "unsafe" cue is safe once fear reversal begins (South et al., 2011). However, in the current study the ASD group did not continue to respond to the CSPlus during the reversal phase more so than either the SAD group or the CON group. Furthermore, the ASD group did not differ significantly on their self-reported fear during the scanner task than either the SAD or CON group. Accordingly, this hypothesis was not supported. This indicates that sample selection, including comorbid SAD, may partially explain differences in perseverative fear. Individuals with ASD are notably rigid in their behaviors, and often have difficulty with learning new tasks. However, comorbid SAD may serve as a protective factor in this group, such that these individuals are sensitive in changes to threat.

In the present study it was hypothesized that neural activation to the CSPlus would be associated with self- and parent-reported measures of anxiety. Results from South and colleagues' (2011) fear conditioning study with adolescents with ASD found a relationship between the conditioned response and social anxiety symptoms. In the current study, there was a significant relationship between parent-reported interference of SAD symptoms on the ADIS and ITG activation for the CSPlus nonsocial condition, with less activation in the ITG associated with greater SAD interference. When examining whether this correlation was driven by any group in particular, no relationship was observed between groups. Both the SAD group and ASD group demonstrated significantly decreased activation of the ITG,

although the events with which they differed in activation differed. Accordingly, the ITG may be particularly sensitive to how interfering SAD symptoms are in an adolescent's life.

In addition to hypothesized results, several other significant findings were observed across behavioral and brain responses. Behaviorally, parents of children with ASD reported their child's anxiety as more impairing than parents of children with SAD. However, adolescents with ASD reported their anxiety as less severe compared to adolescents with SAD without ASD. This finding is consistent with prior literature, which suggests that children with ASD show poor agreement on diagnostic measures of anxiety with parents and clinicians (Storch et al., 2012). In particular, adolescents with ASD tend to underreport psychiatric symptoms (Mazefky, Kao, & Oswald, 2011). Discrepancies between parent and child report in ASD are hypothesized to relate to reduced insight regarding internal states and symptoms of anxiety within children with ASD (Storch et al., 2012). Greater report of symptom interference in parents of children with ASD may due to the increased stress parents of children with ASD feel generally, which was applied specifically to social impairment in this study.

Results of the behavioral responses within the scanner largely did not reveal significant differences between groups at each time point that ratings were collected. This indicates that regardless of diagnostic status, teens reported the same subjective experience of the conditioning and reversal tasks. Notably, the ASD group reported that they found the second face significantly more unpleasant at baseline compared to the SAD group. This finding may be attributable to fatigue in the ASD group at looking at faces. Alternatively, it may reflect discomfort in the SAD group at reporting finding a peer's face as more unpleasant on the first rating. Additionally, the ASD group reported finding the first oval as significantly more pleasant than the CON group. This finding may reflect research that individuals with ASD find nonsocial stimuli more rewarding than social stimuli.

With regard to imaging findings, the clinical groups were found to differ from the Control group during the fear acquisition when comparing social stimuli to nonsocial stimuli. In particular, the ASD and SAD group both demonstrated increased activation in the visual cortex compared to typically

developing adolescents. Activation in the visual cortex is common in fear conditioning tasks that utilize visual stimuli, according to a meta-analysis of fear conditioning studies (Sehlmeyer et al., 2009). Activation within the inferior visual cortex, such as that observed in this study, within the context of fearful stimuli, is associated with emotional intensity (Bradley et al., 2003). Specifically, Bradley et al. (2003) found in an fMRI study that the more salient and intense images were (e.g., violent images), the greater activation was observed in the visual, or occipital, cortex. Typically developing participants in this study did not demonstrate heightened activation to less intense images (e.g., angry faces). The authors argued that this type of activation is defensive, survival-based attention. Notably, participants in the present study viewed images of angry faces paired with a scream sound. Typically developing participants did not demonstrate increased activation of the inferior occipital cortex, while participants in both clinical groups did demonstrate this activation. This indicates that both patient populations found the fear conditioned stimuli salient, and directed survival-salient attention toward these stimuli.

Adolescents with SAD demonstrated significant activation in the middle frontal gyrus during nonsocial fear conditioning for the CSPlus compared to the CSMinus. This result is somewhat consistent with emerging literature that the development of anxiety disorders develops from subcortical regions (e.g., amygdala) to later-maturing regions, such as prefrontal cortex (Shechner, Hong, Britton, Pine, & Fox, 2014; Lau et al., 2011). However, the ASD group did not also demonstrate this activation. This indicates that SAD alone may have different mechanisms for fear acquisition compared to ASD with comorbid SAD.

Both the SAD group and CON group demonstrated activation in the occipital cortex during the CSPlus in the nonsocial condition compared to the social condition. This indicates that the SAD group encoded fear responses for both conditions, regardless of social salience. The CON participants did not evidence significant activation in this region during the social condition, which indicates that they likely did not find the social condition to be particularly aversive or salient. Conversely, the ASD group only

evidenced greater activation in this region during the social condition, which indicates this group may be particularly vulnerable to social threats.

The SAD group additionally demonstrated greater activation in the precuneus. Although activation in this region was not hypothesized to respond to fear conditioning, it has been identified as sensitive to fear conditioning tasks in prior work. In particular, the precuneus seems responsive to social salience generally, and aversive conditioning more specifically (Pizagalli, Greischar, & Davison, 2003). Dunsmoor et al. (2007) found that precuneus activation increased as the contingency between the CS-UCS increased, such that as pairing of the CS with the UCS increased so did the activation of the precuneus. Additionally, activation of the precuneus during fear conditioning is associated with generalization of the feared stimulus (Lissek et al., 2014). Accordingly, the present results indicate that social anxiety increases sensitivity to the pairing of the CS with the UCS and may develop a generalized fear for the social stimulus compared to the nonsocial stimulus.

In addition to the role of the precuneus in fear conditioning paradigms, this brain region considered a hub of the Default Mode Network (DMN; Utevsky, Smith & Huettel, 2014), and is hypothesized to relate to self-referential processing (Cavanna & Trimble, 2006). The involvement of the precuneus in the SAD group during the social fear acquisition provides a potential neural mechanism to harness for treatment of SAD. Previous literature indicates that mindfulness and meditation alters the functioning of the DMN. For example, in an fMRI study of individuals who meditate often, decreased activation in DMN activation generally, and the precuneus specifically, was associated with more practice with meditation (Garrison, Zeffiro, Scheinost, Constable, Brewer, 2015). This effect was observed both at rest as well as during tasks.

Although the groups were similar on almost all ratings of fear for the stimuli, neural patterns conveyed a different story and indicate that there are group differences in fear processing. Participants with ASD and SAD demonstrated greater activation in brain regions associated with heightened emotional salience and fear-sensitivity, despite not self-reporting this. This is concordant with prior

literature that participants with ASD report unpleasant stimuli as more pleasant (Shalom et al., 2006) and SAD participants show heightened physiological arousal despite similar self-report data compared to their peers (Britton et al., 2013). These results indicate that utilizing neural responses provides information above and beyond self-report alone. Furthermore, results indicate that different neural pathways may exist for the development of comorbid ASD and SAD compared to SAD alone. Differences in neural underpinnings may necessitate different treatment approaches. For example, in ASD, treatments that are not reliant on higher-order processing of threat are likely best, due to a reduction in connections observed between the PFC and lower-order neural structures, such as the amygdala. This is consistent with LeDoux & Pine (2016), who report that activation of lower-level neural processes without top-down activation displayed is suggestive of an approach that does not rely on cognitive therapy. Instead, these patient may respond better to exposure-based therapy without emphasis on cognitive restructuring may be best practice, or a combination of exposure-based techniques and mindfulness-based interventions. For SAD uncomplicated by ASD, a mindfulness-based approach may be preferable, due to observed dysregulation in the precuneus, a central hub of the DMN that is sensitive to meditation. Additionally, these results may hold insight into psychopharmaceutic approaches.

This study should be viewed in light of limitations. Data from the fMRI were unable to be analyzed from almost half of the ASD group, resulting in underpowered group means. Additionally, an extensive clinical interview was not administered to participants, which means that control participants may have met diagnostic criteria for other forms of anxiety, and clinical groups may have had a diagnosis of a clinical disorder other than SAD or ASD as their primary diagnosis.

In sum, it should be noted that hypothesized brain regions, the amygdala and prefrontal cortex, were not significantly active in the current study as a result of condition. Prior research has demonstrated atypical amygdala responsiveness in ASD, such that participants with ASD did not differentiate between the CSPlus and the CSMinus (Top et al., 2016). The present study also did not

observe greater amygdala activation across any condition. Although the amygdala is primarily indicated in work on fear acquisition and expression, the development and manifestation of fear exists even in persons who have lesions to the amygdala. For example, participants with damage to their amygdala self-report comparable levels of fear responses to controls (Anderson & Phelps, 2002). Additionally, patients with bilateral amygdala damage demonstrate expected panic responses in a CO2 inhalation study (Feinstein et al., 2013). Together, this indicates that the amygdala alone is not solely responsible for development and expression of fear. Instead, a widespread fear network exists, with encoding across brain regions (LeDoux & Pine, 2016).

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**Table 1.** Participant demographics

| | ASD (*n* = 17) | ASD | TD (n = 20) | TD | SAD (n=20) | SAD |
|---|---|---|---|---|---|---|
| | **Mean (*SD*)** | **Range** | **Mean (*SD*)** | **Range** | **Mean (*SD*)** | **Range** |
| **Age (in years)** | 14.6 *(2.09)* | 12-17 | 14.2 *(1.77)* | 12-17 | 14.4 *(1.82)* | 12-17 |
| **FSIQ-2** | 104.35 *(15.24)* | 85-129 | 116.4 *(12.56)* | 89-139 | 109.5 *(12.16)* | 92-132 |
| **SRS-2** | 77.5 *(9.47)* | 58 - 90 | 45.1 *(5.90)* | 38 - 59 | 56.6 *(10.6)* | 42 - 79 |
| **LSAS** | | | | | | |
| **Total Score** | 45.5 *(31.2)* | 4 - 104 | 24.4 *(17.2)* | 3-69 | 61 *(30.7)* | 17-121 |
| **Fear** | 23.1 *(15.4)* | 2-51 | 12.2 *(9.22)* | 0-35 | 33.9 *(14.8)* | 12-63 |
| **Avoidance** | 22.4 *(16.7)* | 1-55 | 12.2 *(8.50)* | 2-34 | 27.1 *(16.5)* | 5-58 |
| **ADIS (parent)** | | | | | | |
| **Interference** | 6.12 *(1.32)* | 4-8 | 0.8 *(1.06)* | 0-4 | 4.45 *(1.67)* | 1-7 |
| **CSR** | 5.24 *(1.25)* | 3-8 | 0.65 *(0.875)* | 0-3 | 4.55 *(1.36)* | 1-6 |
| **ADIS (child)** | | | | | | |
| **Interference** | 2.88 *(3.12)* | 0-8 | 1.8 *(1.53)* | 0-5 | 4 *(2.18)* | 0-8 |
| **CSR** | 3.29 *(2.37)* | 0-8 | 0.9 *(0.97)* | 0-3 | 4.75 *(1.37)* | 1-6 |

**Table 2.** Participants included in fMRI analyses compared to those not included

| | Gender | Age | FSIQ | SRS-2 | LSAS Total | ADIS composite |
|---|---|---|---|---|---|---|
| | **M:F** | **Mean** | **Mean** | **Mean** | **Mean** | **Mean** |
| **ASD excluded** | 6:2 | 14.13 | 104.75 | 75.75 | 48.25 | 5.38 |
| **ASD included** | 7:2 | 15 | 104 | 79 | 43.1 | 4.89 |
| ***t*-test: (*p*)** | -0.13 (0.90) | -0.84 (0.42) | 0.09 (0.9) | -0.70 (0.5) | 0.33 (0.75) | 0.92 (0.38) |
| | | | | | | |
| **SAD excluded** | 0:2 | 13 | 108 | 50 | 55.5 | 3 |
| **SAD included** | 7:12 | 14.6 | 109.67 | 57.28 | 61.61 | 5.27 |
| ***t*-test: *t* (*p*)** | -3.29 (0.004) | -1.48 (0.32) | -0.39 (0.72) | -2.82 (0.01) | -0.16 (0.89) | -2.24 (0.25) |
| | | | | | | |
| **CON excluded** | 3:4 | 15.3 | 112 | 44.86 | 27.29 | 0.85 |
| **CON included** | 8:5 | 13.6 | 118.78 | 45.23 | 22.85 | 1.2 |
| ***t*-test: *t* (*p*)** | -0.76 (0.46) | 2.29 (0.03) | -.098 (0.35) | -0.17 (0.87) | 0.48 (0.64) | -0.82 (0.43) |

**Table 3.** ANOVA results for self-and parent-report and clinician interview

| Measure | *df* | SS | MS | *F* | *p* |
|---|---|---|---|---|---|
| **LSAS** | | | | | |
| **Total Score** | 2 | 13491 | 6746 | 9.321 | 0.0003 |
| **Fear** | 2 | 4731 | 2365.4 | 13.28 | >.0001 |
| **Avoidance** | 2 | 2295 | 1147.3 | 5.629 | 0.006 |
| **ADIS (parent)** | | | | | |
| **Interference** | 2 | 278.8 | 139.38 | 73.85 | >.0001 |
| **CSR** | 2 | 234.95 | 117.47 | 85.08 | >.0001 |
| **ADIS (child)** | | | | | |
| **Interference** | 2 | 73.54 | 36.77 | 7.466 | 0.001 |
| **CSR** | 2 | 150.8 | 75.43 | 28.47 | >.0001 |
| **ASEBA** | | | | | |
| **CBCL Anxiety** | 2 | 3263 | 1631.5 | 18.79 | >.0001 |
| **YSR Anxiety** | 2 | 801.5 | 400.7 | 9.2 | 0.0003 |

**Table 4.** Activation Table

| Condition | Event | Group | X | Y | Z | Mean | Region |
|---|---|---|---|---|---|---|---|
| Social>Nonsocial | CSPlus | ASD | -34 | -78 | 30 | 65.2 | Lateral Occipital Cortex |
| Social>Nonsocial | CSPlus | ASD | -58 | -64 | -14 | 53.5 | Lateral Occipital Cortex |
| Social>Nonsocial | CSPlus | SAD | -6 | -62 | 22 | 44.1 | Precuneus Cortex |
| Social>Nonsocial | CSPlus | SAD | -42 | -70 | 42 | 45.8 | Lateral Occipital Cortex |
| Nonsocial>Social | CSMinus | CON | -46 | -76 | 4 | 32.3 | Lateral Occipital Cortex |
| Nonsocial>Social | CSMinus | SAD | 48 | -84 | -4 | 26.9 | Lateral Occipital Cortex |
| Social>Nonsocial | CSPlus>CSMinus | ASD | -60 | -56 | -16 | 41.5 | Inferior Temporal Gyrus |
| Social>Nonsocial | CSPlus>CSMinus | ASD | -34 | -64 | 60 | 65.5 | Lateral Occipital Cortex |
| Social>Nonsocial | CSPlus>CSMinus | SAD | -8 | -34 | 64 | 28.4 | Postcentral Gyrus |
| Social>Nonsocial | CSPlus>CSMinus | SAD | 22 | -24 | 6 | 19.9 | Right Thalamus |
| Social>Nonsocial | CSPlus>CSMinus | SAD | -54 | -68 | -24 | 35.9 | Lateral Occipital Cortex |
| Nonsocial>Social | CSPlus>CSMinus | *F*-test | -28 | -64 | 52 | -- | Lateral Occipital Cortex |
| Nonsocial>Social | CSPlus>CSMinus | *F*-test | -62 | -56 | -14 | -- | Inferior Temporal Gyrus |
| Social>Nonsocial | CSPlus-Reversed | SAD | -46 | 12 | 30 | | Middle Frontal Gyrus |

**Figure 1.** Paradigm schematic for conditions and self-rating.

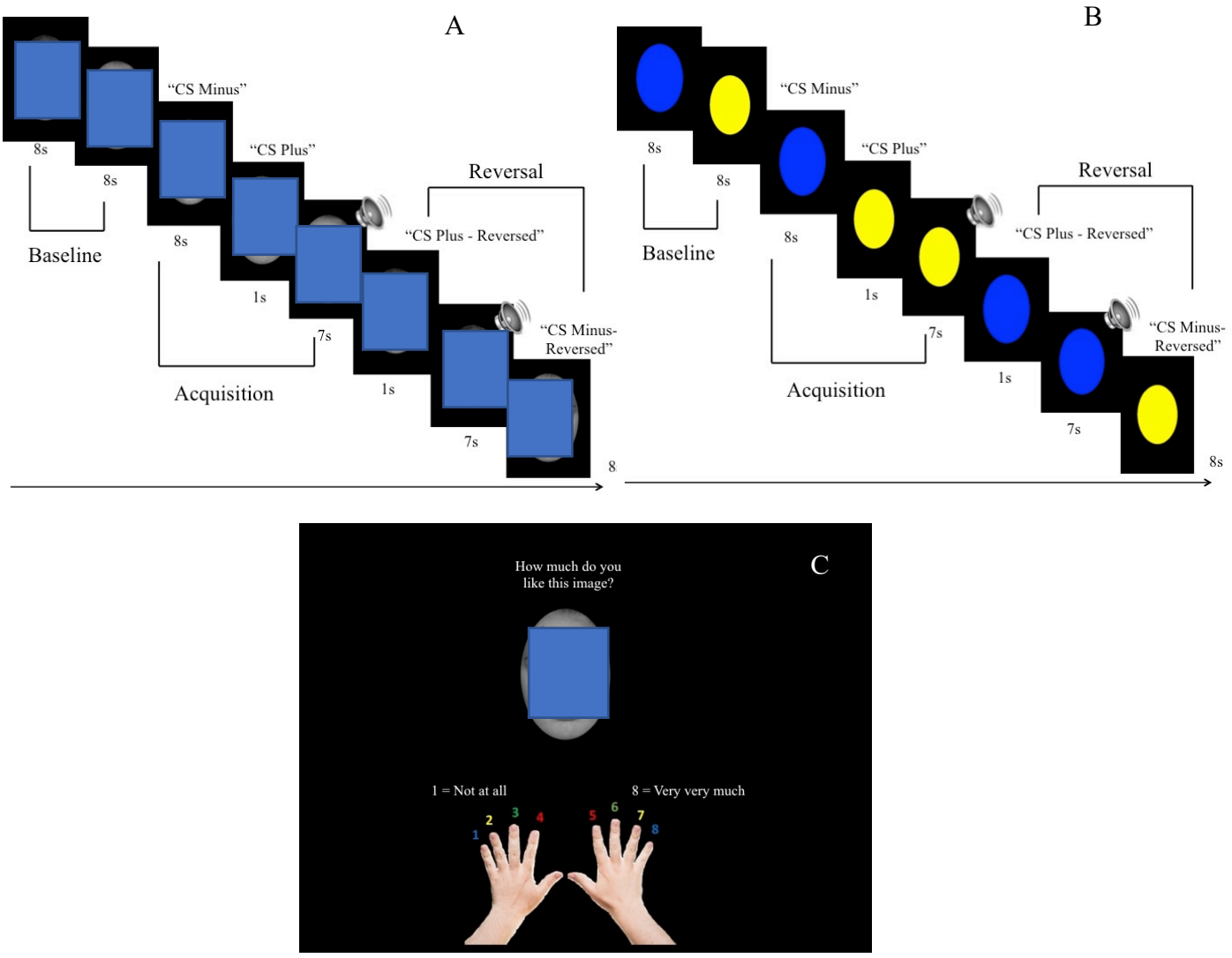


Figure 1. 1A. Represents the social condition, such that participants learn a pairing for one adolescent face with a scream and another that remains neutral and then a reversal of these contingencies. 1B. Represents the nonsocial condition, with colors paired with a startle probe sound or no sound and then a reversal of these contingencies. 1C. Example of self-rating of like, pleasant, unpleasant, and fear, presented 5 times throughout each condition for each stimulus. Pictures of social stimuli covered in blue boxes because the Virginia Tech Graduate School would not allow the display of these images, despite agreement by parents and children to be featured in scientific publication.

**Figure 2.** LSAS ratings

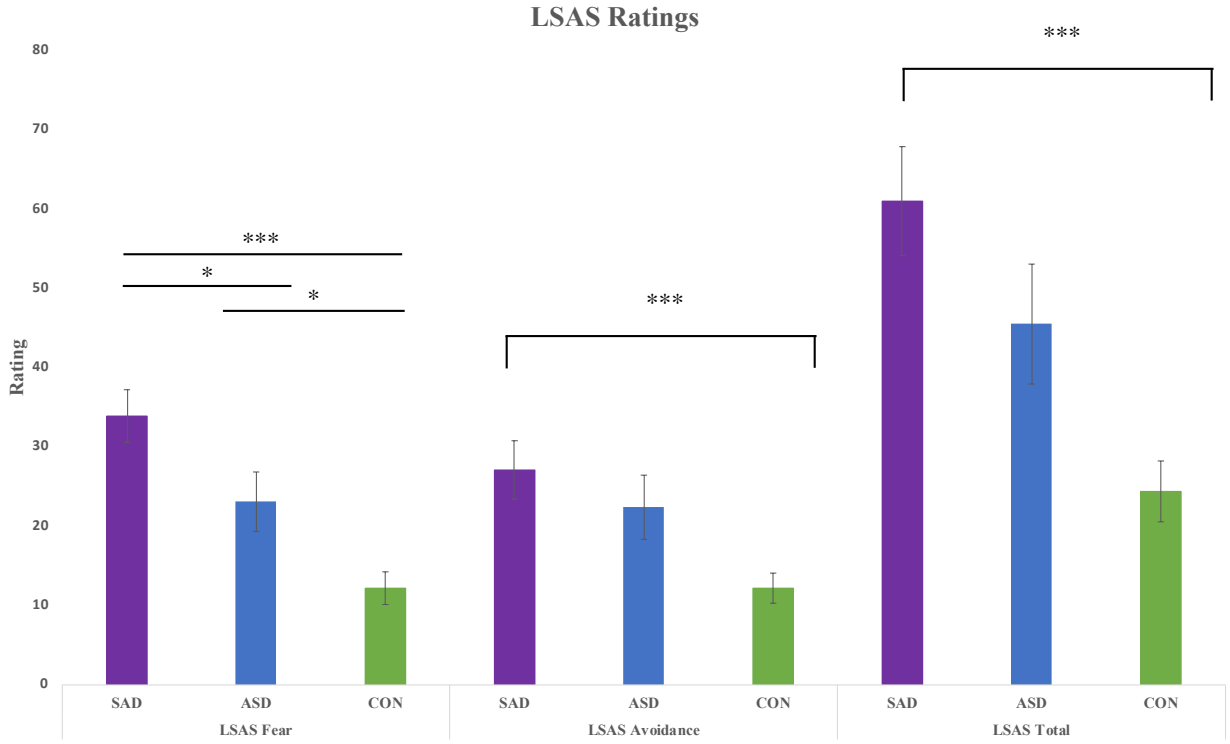


**Figure 2.** Bar graph of mean LSAS scores reported by child. Total score and subscale scores are represented. Significant differences between groups is shown with *** as less than 0.001, and * as less than 0.05.

**Figure 3.** ADIS scores by group

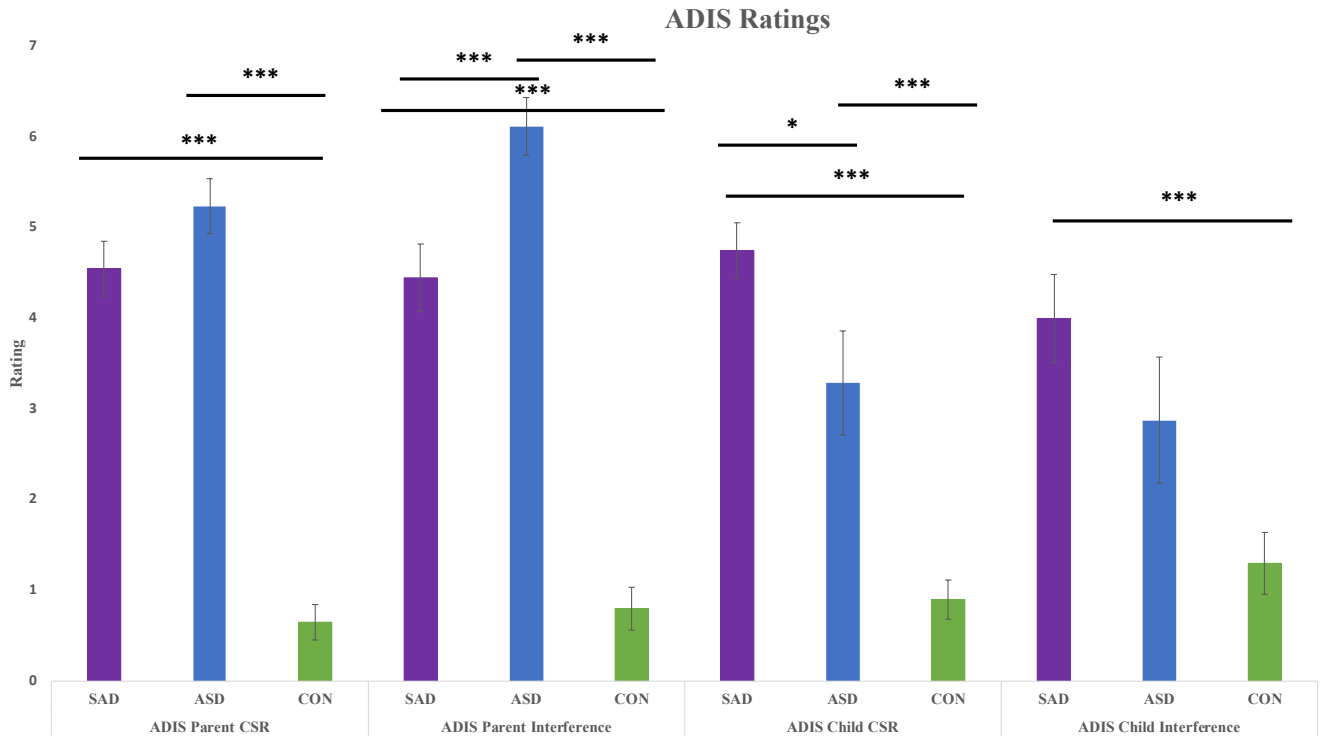


**Figure 3**. Bar graph of mean ADIS scores reported by parent and child. CSR scores represent interviewer ratings, and Interference scores represent parent or child ratings of severity of overall symptoms of SAD. Significant differences between groups is shown with *** as less than 0.001, and * as less than 0.05.

**Figure 4.** Significant activation across groups for CSPlus>CSMinus

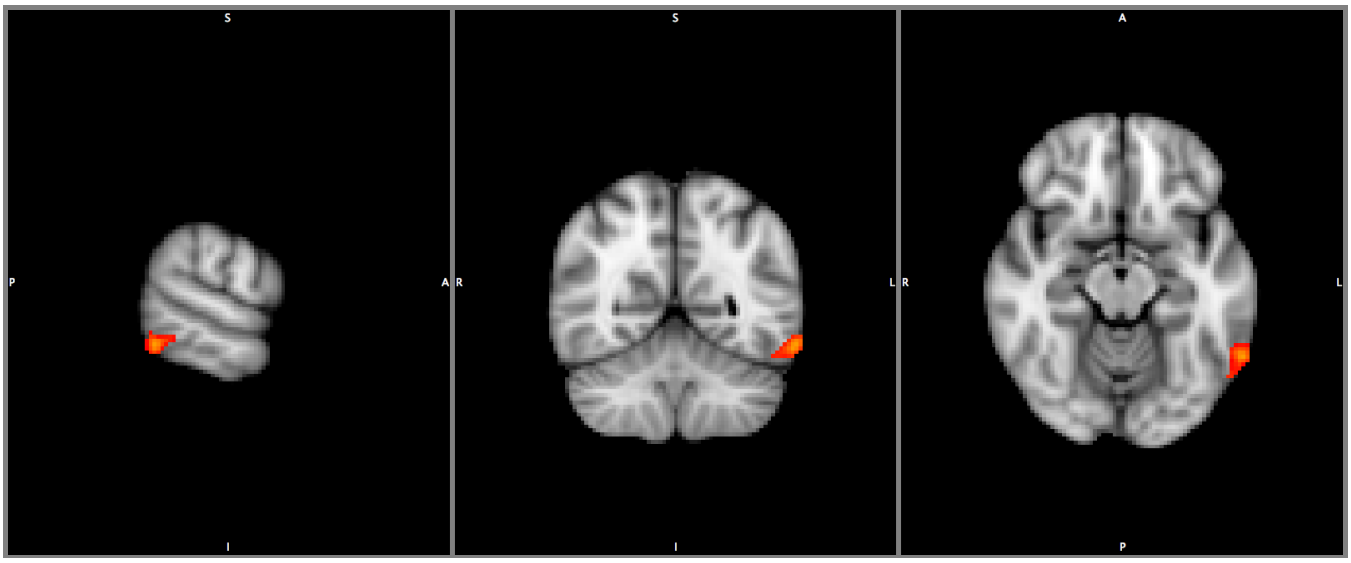


Figure 4. Significant activation of the ITG was observed for the 3 Group (ASD, SAD, CON) X 2 Event (CSPlus, CSMinus) X 2 Condition (Social, Nonsocial) ANOVA.

**Figure 5.** Significant activation across groups for CSPlus>CSMinus in Social compared to nonsocial condition

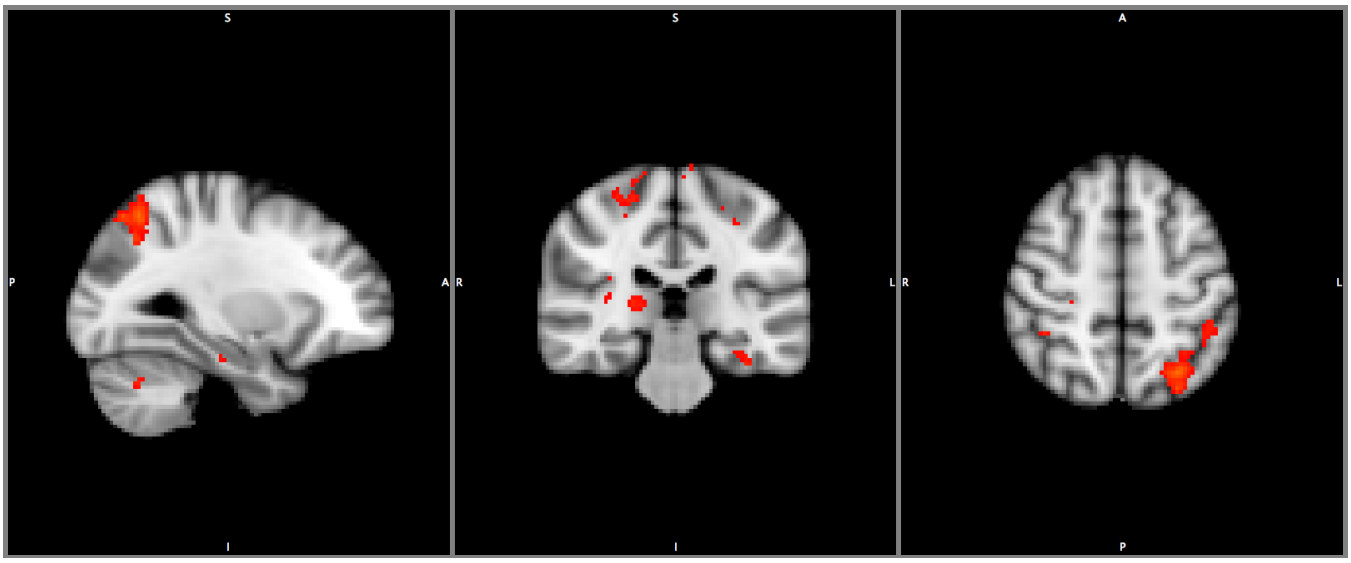


Figure 5. Significant activation of the lateral occipital lobe was observed for the 3 Group (ASD, SAD, CON) X 2 Event (CSPlus, CSMinus) X 2 Condition (Social, Nonsocial) ANOVA.

**Figure 6.** Bar graph for Mean ITG activation by Group, Event, and Condition.

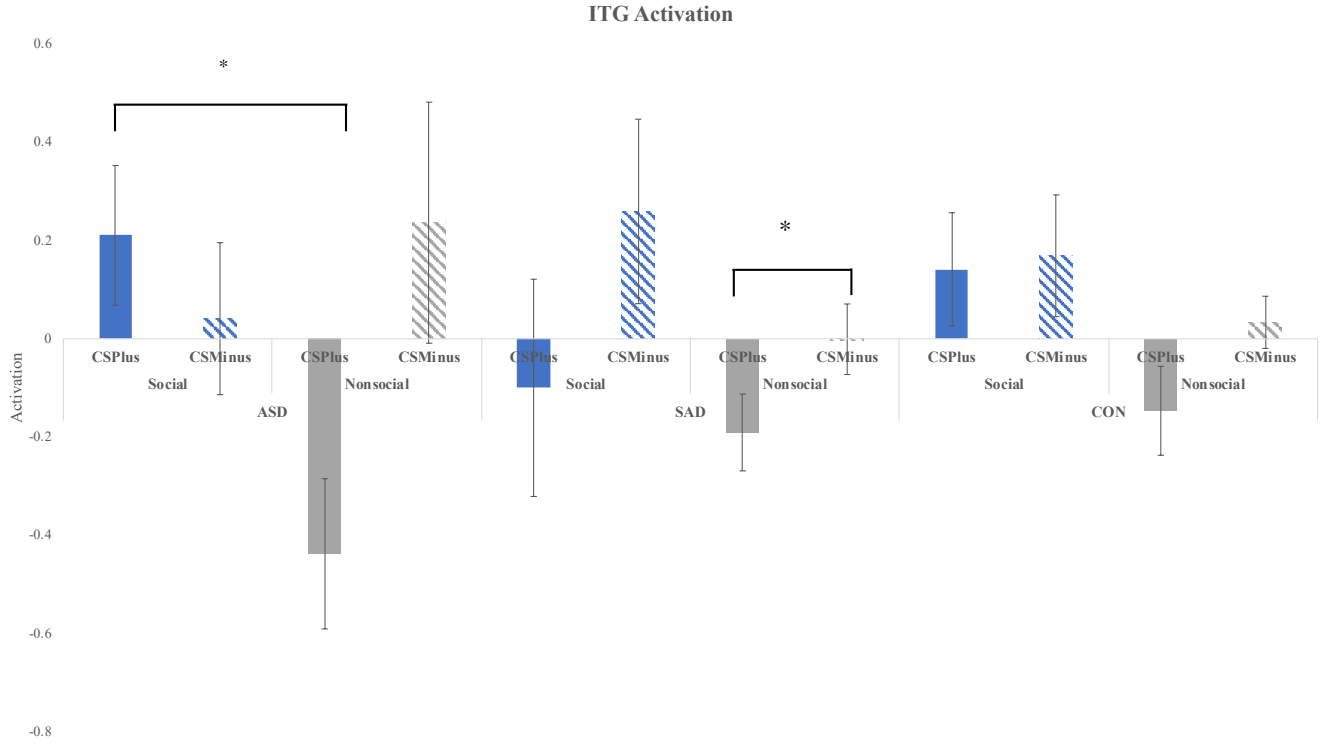


Figure 6. Bar graph depicting activation in the Inferior Temporal Gyrus for Event (CS Plus, CS Minus) and Condition (Social, Nonsocial). Social condition results are shown in blue, and nonsocial condition results are shown in grey. CSPlus event results are shown in solid, and CSMinus events are shown in stripes. Significant results are depicted with a line, with (*) denoting $p<0.05$.

**Figure 7.** Scatterplot of correlation for CSPlus event, nonsocial condition and parent interference ratings.

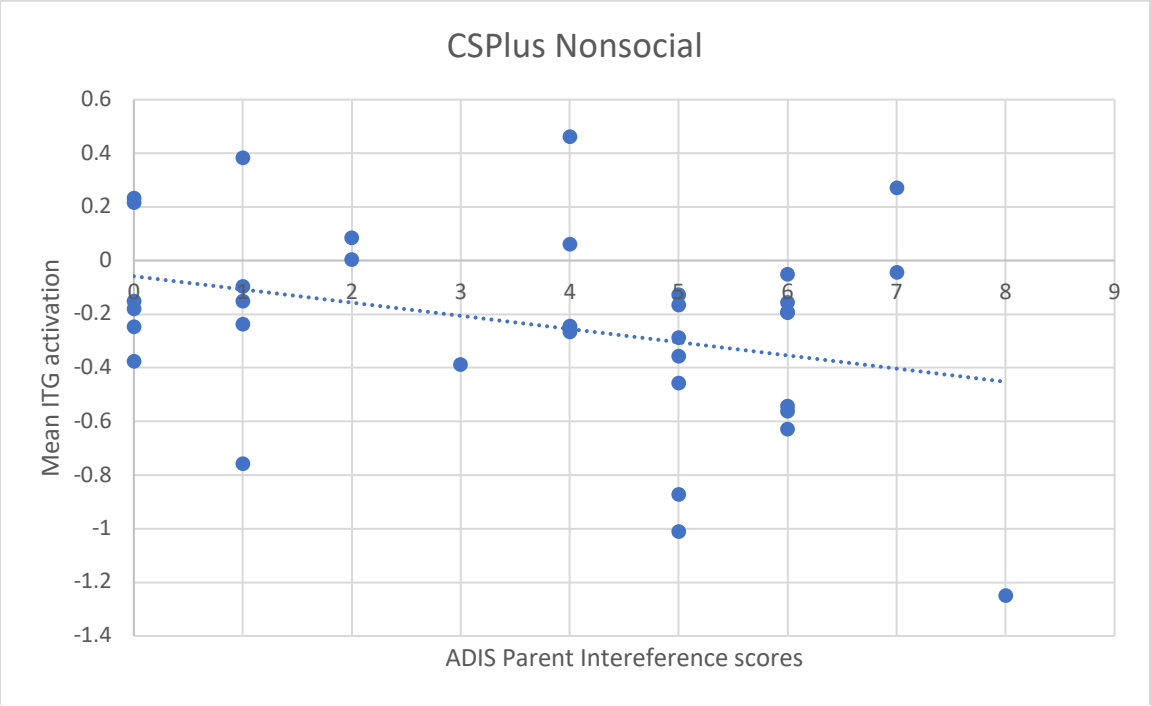


Figure 7. Parent rated interference on ADIS SAD module and Mean ITG activation during CSPlus Nonsocial condition ($p$ = 0.04, $r$= -0.33).

**Figure 8.** Bar graph for Like ratings in fMRI scanner.

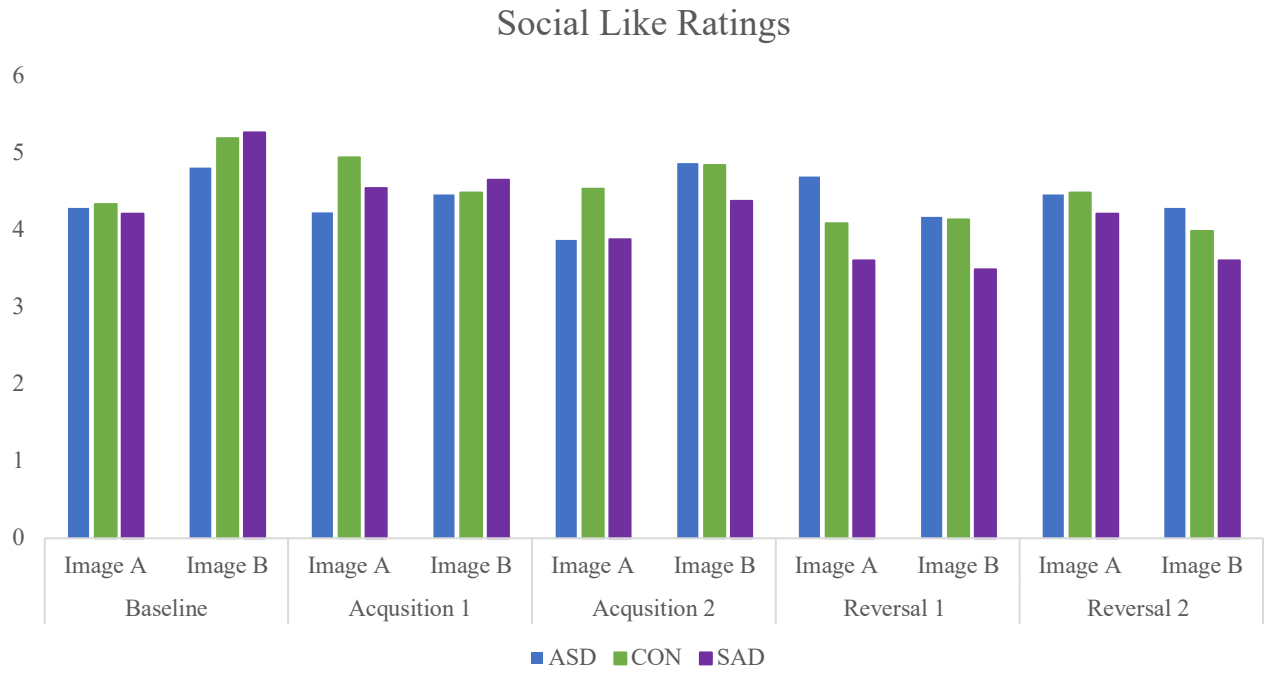


Figure 8. Self-report for how much participants liked faces across time points (Baseline, Acquisition ratings, reversal ratings) by group. No significant group differences were observed.

**Figure 9.** Bar graph for Pleasant ratings in fMRI scanner by Group.

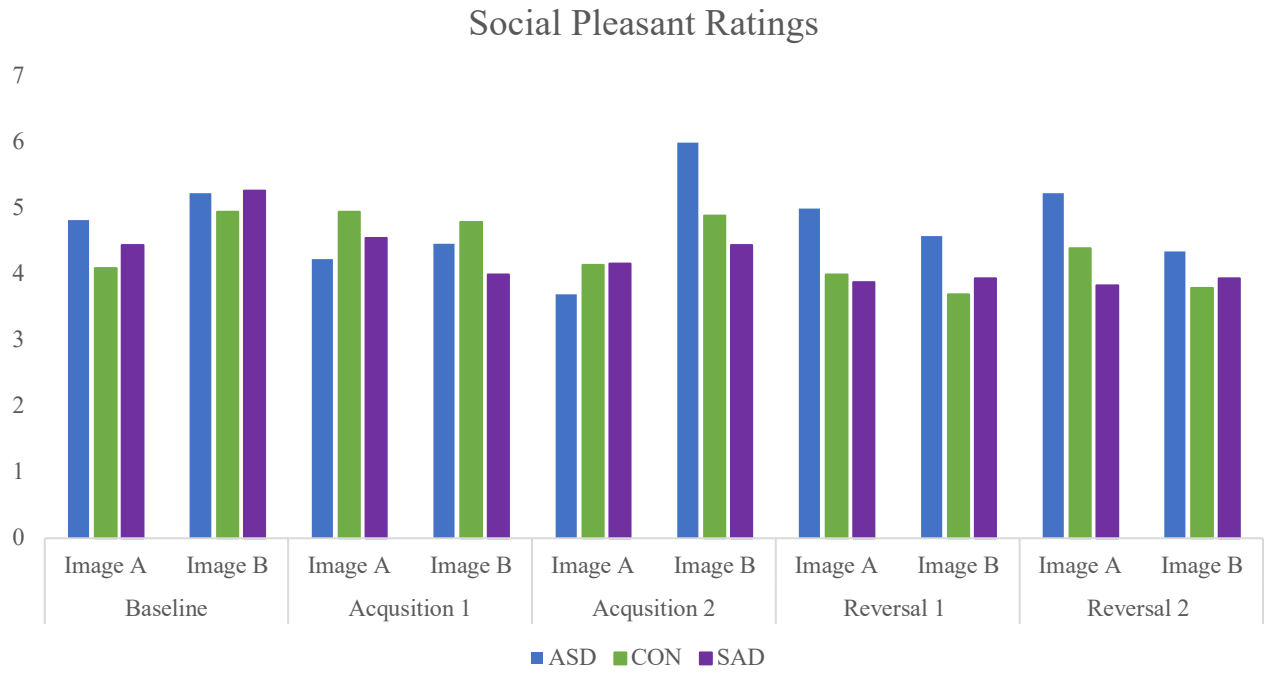


Figure 9. Self-report for how pleasant participants liked faces across time points (Baseline, Acquisition ratings, reversal ratings) by group. No significant group differences were observed.

Figure **10.** Bar graph for Social Unpleasant ratings in fMRI by Group.

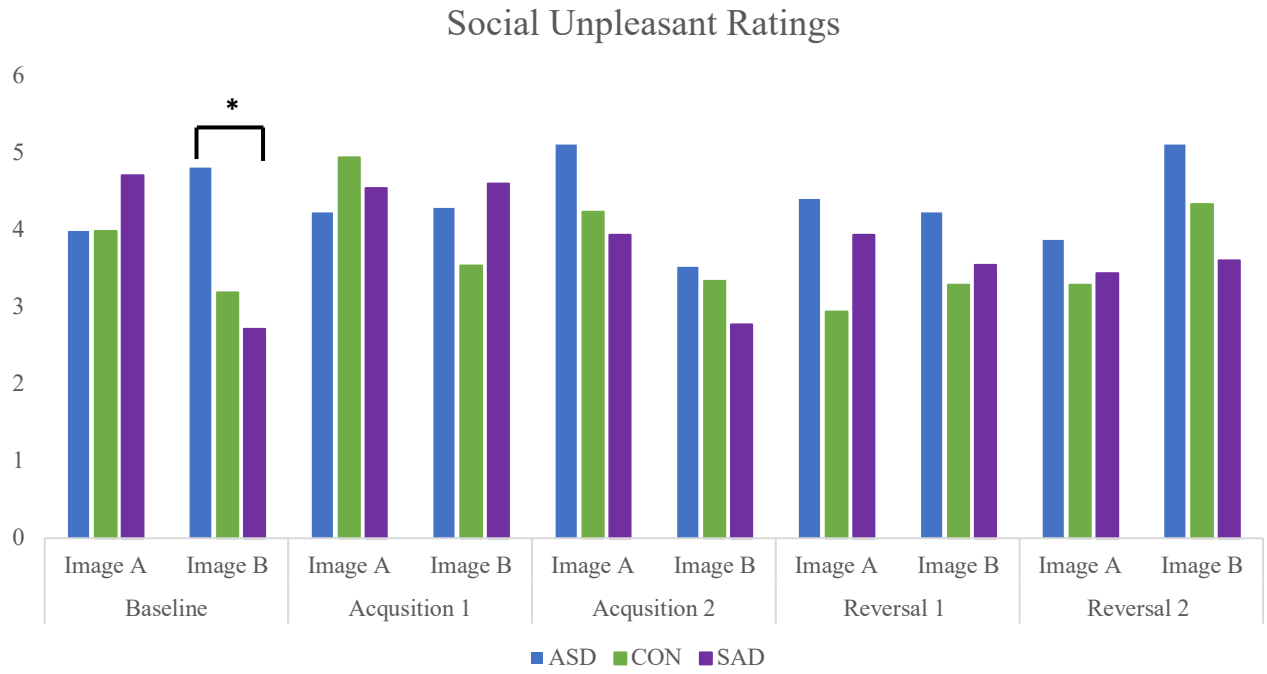


Figure 10. Self-report for how unpleasant participants found faces across time points (Baseline, Acquisition ratings, reversal ratings) by group. A significant effect was observed for how unpleasant participants found the second face at Baseline ($F(2,52) = 3.208$, $p = 0.049$), such that the ASD group found the second face significantly more unpleasant than the SAD group ($p = 0.049$), denoted by *.

Figure **11.** Bar graph for Fear ratings in Social condition by Group.

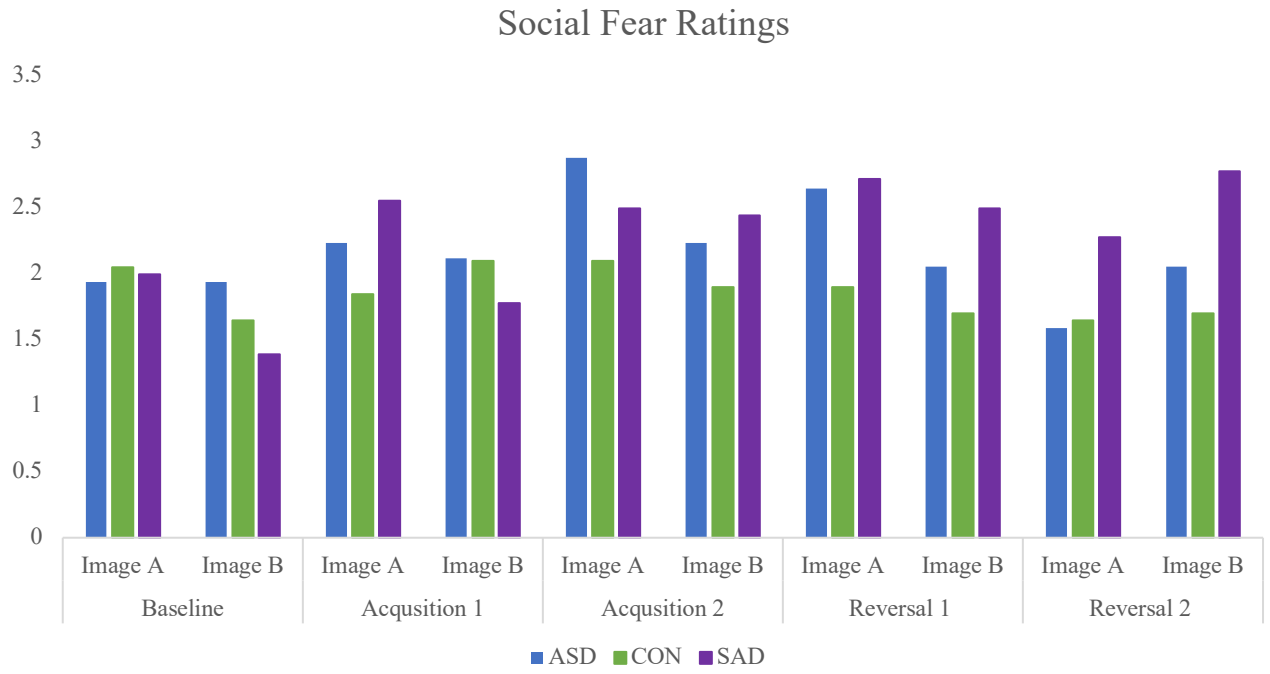


Figure 11. Self-report for how afraid participants were of faces across time points (Baseline, Acquisition ratings, reversal ratings) by group. No significant differences were observed

**Figure 12.** Bar graph for Like ratings in fMRI scanner during Nonsocial condition

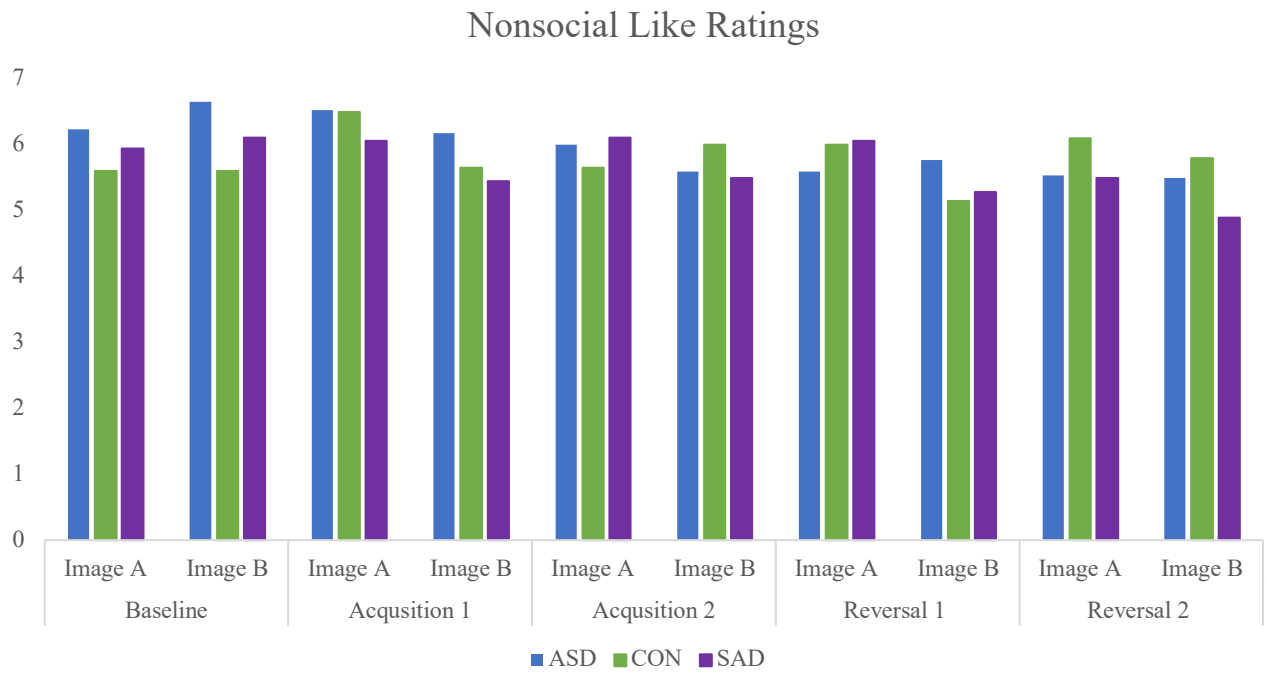


Figure 12. Self-report for how much participants liked ovals across time points (Baseline, Acquisition ratings, reversal ratings) by group. No significant differences were observed.

**Figure 13**. Bar graph for Pleasant ratings during fMRI in Nonsocial condition.

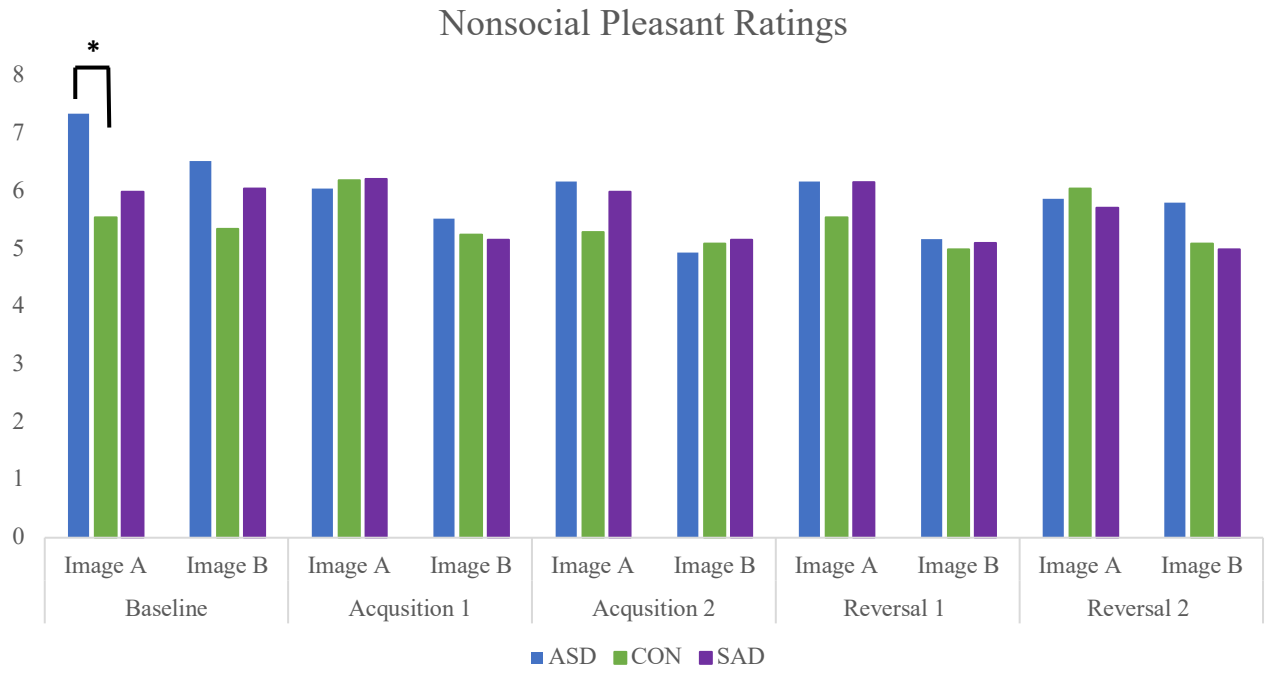


Figure 13. Self-report for how pleasant participants liked ovals across time points (Baseline, Acquisition ratings, reversal ratings) by group. There was a significant group differences for the Baseline condition ($F(2,52) = 3.56$, $p = 0.036$), such that the ASD group reported finding the first oval as significantly more pleasant than CON group ($p = 0.03$), denoted by *.

**Figure 14.** Bar graph for Unpleasant ratings in fMRI during Nonsocial condition.

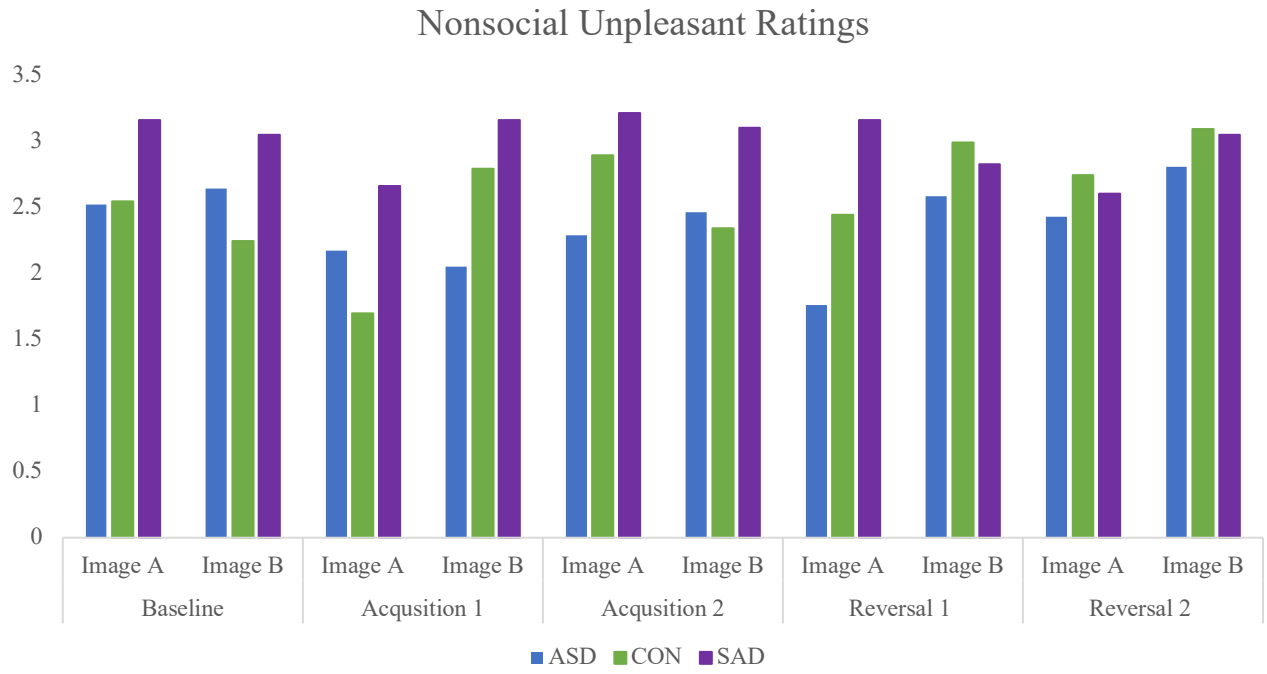


Figure 14. Self-report for how unpleasant participants liked ovals across time points (Baseline, Acquisition ratings, reversal ratings) by group. No significant differences were observed.

**Figure 15.** Bar graph for Fear ratings during fMRI for Nonsocial condition.

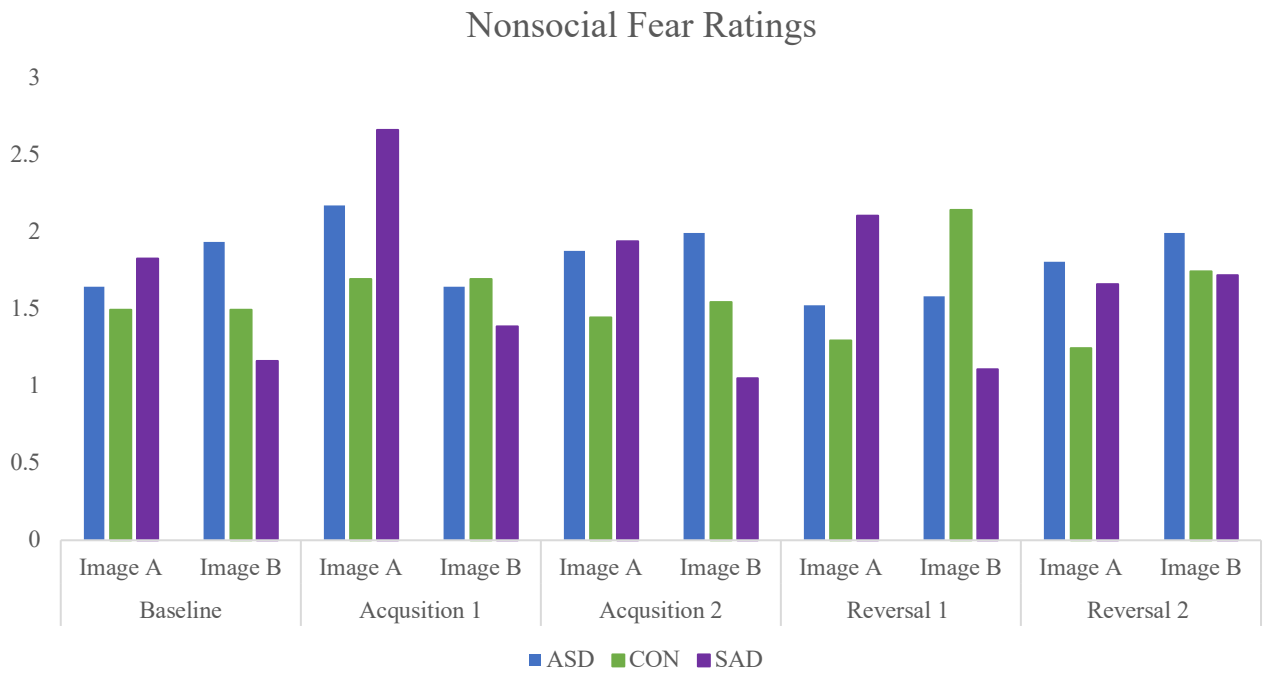


Figure 15. Self-report for how much participants were afraid of ovals across time points (Baseline, Acquisition ratings, reversal ratings) by group. No significant differences were observed.