Larger Amygdala Volume Relates to Social Anxiety in Youth with Autism Spectrum Disorders

by

Sarah L. Alberti

A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of Bachelor of Sciences

with Honors in Biopsychology, Cognition, and Neuroscience from the

University of Michigan

2013

Advisor: Dr. Christopher Monk

Abstract

Abnormalities in the amygdala, a structure centrally associated with emotional processing, have been suggested as a neurobiological substrate of the socio-emotional deficits observed in autism spectrum disorders (ASD). Moreover, youth with ASD are at increased risk for anxiety disorders, possibly due to socio-emotional impairments. Using measurements from magnetic resonance imaging (MRI), as well as the Multidimensional Anxiety Scale for Children (MASC), I compared amygdala volume and anxiety levels between youth with ASD and healthy controls, aged 8-19 years. Relative to controls, participants with ASD displayed significantly larger bilateral amygdala volumes, as well as higher levels of social and separation anxiety. A significant interaction between group, social anxiety, and amygdala volume was present within the ASD group, driven by a positive correlation between social anxiety scores and bilateral amygdala volumes. No relationships between amygdala volume and anxiety were found in controls. These results suggest that within ASD, larger amygdala volume is associated with a higher degree of social anxiety.

Keywords: adolescent, anxiety, amygdala, autism, MRI

Larger Amygdala Relates to Social Anxiety in Youth with Autism Spectrum Disorders

Autism Spectrum Disorders (ASD) are associated with impaired socio-emotional understanding, reciprocation, and communication (APA, 2000). It has been suggested that abnormalities in the amygdala, a structure regarded as an underlying component to socio-emotional cognition, play a necessary role in the social deficits observed in ASD (Baron-Cohen et al., 2000).

Amygdala Abnormalities

Numerous MRI studies have found abnormal amygdala structure in autism. Evidence of the extent of these abnormalities, however, has remained relatively inconsistent. Previous findings suggest that young children with ASD evince significantly larger amygdala volume (Nordahl et al., 2012; Sparks et al., 2002). Similar results have been found in adolescents, as well as in adults with high-functioning autism (Buitelaar, Groen, Teluij, & Tendolkar, 2010; Howard et al., 2000). However, others have found increased amygdala volumes in childhood, which significantly decrease into adolescence (Schumann et al., 2004), and decreased limbic volumes in both adolescents and adults (Aylward et al., 1999). Inconsistencies in previous findings may possibly be due to the various age groups studied, as well as disparate sample sizes (for instance, 85 participants with ASD aging 2-4 in Nordahl et al., 2012; 10 participants with ASD ranging 15.8-40.3 years in Aylward et al., 1999).

Age and Development

Although the amygdala continues to develop throughout the first two decades of life, children with ASD may reach abnormally large volumes, which appear to cease in late childhood and decrease into adolescence and adulthood. (Schumann et al., 2004). Similar growth trajectories have been found in the cortex as well. Courchesne and colleagues (2001) found a

quadratic relationship between cerebral cortical gray matter and age, which was present in controls but much more pronounced in autism. Youth with ASD displayed an initial neural overgrowth, which 'peaked' earlier in childhood and decreased to reach similar, and eventually lower volumes, relative to controls. If there is in fact a nonlinear relationship between amygdala volume and age, anatomical studies performed on youth with restrictive age ranges may yield conflicting results.

Anxiety

Along with neuroanatomical changes, late childhood and adolescence presents a large number of cognitive and social transitions. Vulnerabilities to emotional disorders increase, especially in those with ASD. Anxiety is notably prevalent in ASD, and may worsen throughout adolescence (Kuusikko et al., 2009; Ollendick, Oswald, Scahill, & White, 2009). Although anxiety has long been associated with ASD (Kanner, 1943), little work has investigated the particular types of anxiety common of autism, relative to amygdala structure. Juranek and colleagues (2006) found a positive correlation between general anxiety scores on the Child Behavior Checklist (CBCL; Achenbach, 1991) and right amygdala volume in children with ASD ages 3.7 – 14.7 years. Conversely, Corbett and colleagues (2009) suggested an interaction between smaller amygdala volumes, increased social anxiety, and age group in older children; and Nacewicz and colleagues (2006) have speculated social anxiety as a mediator between decreased amygdala volume and increased social impairments in adolescent and adult males with higher functioning ASD.

Social Anxiety

The prevalence of social anxiety in autism seems logical, given the social nature of ASD impairments. Moreover, increased social anxiety has been found to negatively correlate with

social behavioral impairments in youth with ASD (Bellini, 2004; Kleinhans et al., 2010). And as Ollendick, Oswald, Scahill, and White (2009) have noted, a high risk for developing social anxiety may root from the fact that many individuals with ASD are often uncomfortable or embarrassed about their impaired social functioning. Therefore, social impairments and anxiety may exacerbate one another. Because the distinction between autism-associated social impairments and anxiety-associated social impairments is often ambiguous in ASD, more research must be done on the neuroanatomical predictors of anxiety in ASD, as well as the types of anxiety that commonly occur.

The goal of this study was to characterize the relationship between social anxiety and amygdala structure in autism, relative to other types of anxiety. In order to do this, I compared measurements of anxiety symptoms and amygdala volume in between youth with ASD and healthy controls. Participants ranged in age from 8-19 years: Ages at which amygdala volumes are believed to peak and anxiety is believed to worsen. Amygdala volume was measured using manual tracing methods on MRI, while anxiety was assessed by self-report surveys, measuring four major types of anxiety: physical symptoms, social anxiety, harm avoidance, and separation anxiety.

To investigate the relationships between these factors, I established four main hypotheses. The first prediction was that between groups, participants with ASD would display significantly larger bilateral amygdala volumes relative to controls. Additionally, those in the ASD group would have significantly higher anxiety: Particularly social anxiety. These increased anxiety levels should also correlate positively with amygdala volumes.

Drawing on previous work from Schumann and colleagues (2004), I also created groups of age for participants younger and older than 13 years. I predicted that group differences in

amygdala volume should interact with age group, in that larger amygdala volumes in ASD should remain significant in younger, non-adolescent participants only.

Method

Participants

Ninety-five children and adolescents with ASD and 72 healthy controls participated in this study. Those with ASD were recruited through the University of Michigan Autism and Communication Disorders Center (UMACC), while healthy controls were recruited through advertisements and flyers. All participants with ASD were clinically diagnosed based on the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000), and the Autism Diagnostic Interview-Revised (ADI-R; Le Couteur, Lord, & Rutter, 1994). Twenty-nine participants with ASD and two controls were removed from the sample after scoring < 85 in cognitive functioning. Additionally, one participant with ASD and one control quit the scan due to anxiety, two participants with ASD and three controls were unable to go into the scanner, seven participants with ASD and five controls had untraceable images, and 23 participants with ASD and six controls were excluded due to excessive head movement. The final sample size consisted of 33 individuals with ASD and 55 controls. Participants ranged in age from 8 to 19 years (M = 14.19, SD = 2.76). Youth with ASD had a mean age of 13.53 years (SD = 2.29), while healthy controls had a mean age of 14.59 years (SD = 2.95). There were no significant differences in age, verbal IQ, and non-verbal IQ between groups (see Table 1). The Institutional Review Board approved all procedures, and parents and guardians signed consent/assent forms.

Additionally, 14 individuals with ASD were on psychotropic medications. Three individuals were on selective serotonin reuptake inhibitors (SSRIs), eight were on stimulants for attention-deficit hyperactivity disorder (ADHD), three were on non-stimulant ADHD

medications, two were on anxiolytics, three were on antipsychotics, and four took a combination of medications (see Table 2).

Materials

IQ. To Measure verbal and non-verbal IQ, participants were administered Peabody Picture Vocabulary Tests (PPVT), Differential Ability Scales (DAS), Wechsler Intelligence Scale for Children, Stanford-Binet Intelligence Scales, and Ravens Progressive Matrices. Those who scored < 85 in cognitive functioning were excluded for study.

Anxiety. To measure anxiety, participants completed the Multidimensional Anxiety Scale for Children, a 39-item self-report survey (MASC; March et al., 1997). MASC was chosen due to its noted internal consistency, as well as its discriminative reliability in characterizing four major factors of anxiety: physical symptoms, social anxiety, harm avoidance, and separation anxiety (March et al., 1997; Maddox, Schry, & White, 2012).

Procedure

Participants underwent a structural magnetic resonance imaging (MRI) scan using a 3-T GE Signa. High-resolution sagittal SPGR images were collected, consisting of 110 slices 1.4 mm thick (flip angle = 15°, FOV = 26 cm). All MRI images were saved and organized randomly to ensure I, along with two other raters, was blind to each participant's diagnosis. Participants' left and right amygdalae were identified using manual tracing methods on MRI analyzing software BRAINS2 (University of Iowa, Iowa City, Iowa). We reviewed previous literature, such as that of McMaster and colleagues (2008), to obtain a better understanding of manual tracing methods and the anatomical landmarks of amygdala morphometry. Beginning posteriorly, the amygdala was distinguished between the superiomedial portion of the temporal horn and the medial ambient gyrus (see Appendix). Once each amygdala was traced, the volume was calculated

through BRAINS2. Each rater attained inter-rater reliability coefficients > 0.9 in both hemispheres form 10 randomly selected scans before the remaining 78 images were analyzed.

Results

Amygdala Volume

An independent samples t-test conducted using SPSS (SPSS Inc., Chicago, IL) indicated a significant group difference in left amygdala volume, ($t(86) = -3.58$, $p < .01$), in that participants with ASD displayed larger volumes ($M = 1.30$, $SD = .27$), than controls ($M = 1.12$, $SD = .19$). There was a similar group difference in the right amygdala, ($t(86) = -3.23$, $p < .01$), where again, the ASD group displayed significantly larger volumes ($M = 1.32$, $SD = .26$), compared to controls ($M = 1.16$, $SD = .22$; see Table 1, Figure 1).

Anxiety

Another independent samples t-test revealed significant group differences in social anxiety ($t(76) = -2.92$, $p < .01$) and separation anxiety scores ($t(76) = -3.04$, $p < .01$). Participants in the ASD group had higher social anxiety scores ($M = 11.18$, $SD = 6.46$), than controls ($M = 7.50$, $SD = 4.60$). Additionally, those with ASD had separation anxiety scores ($M = 8.36$, $SD = 4.60$), relative to controls ($M = 5.52$, $SD = 3.54$; see Table 1, Figure 2).

There was a significant interaction between group and social anxiety scores in predicting left ($F(2, 75) = 11.34$, $p < .01$), and right amygdala volumes ($F(2, 75) = 7.04$, $p < .01$; see Table 3). This interaction was driven by a positive correlation between social anxiety and left amygdala volume ($r = .455$, $p < .05$; see Figure 4), as well as right amygdala volume ($r = .402$, $p < .05$) in the ASD group. Thus, participants with higher levels of social anxiety were more likely to have larger amygdala volumes. Separation anxiety, however, did not share a significant relationship with either left ($r = .334$, $p = .082$) or right amygdala volumes ($r = .256$, $p = .188$). No significant

correlations between amygdala volume and anxiety were evident in controls (see Table 4, Figure 3).

Age

Age did not correlate significantly with left amygdala volume, right amygdala volume, or any anxiety score. However, I created age groups to further investigate relationships between group, social anxiety, and amygdala volume within late childhood and adolescence separately. Participants were split into age groups younger than 13 years (n = 30) and older than 13 years (n = 57). I then ran an independent samples t-test to reassess group differences in amygdala volume within each age group. In younger participants, those with ASD had significantly larger left amygdala volumes (M = 1.30, SD = 0.31), relative to controls (M = 1.10, SD = 0.16; $t(29) = -2.272$, $p < .05$). In the older age group, participants with ASD also had larger left amygdala volumes (M = 1.30, SD = 0.24), ($t(54) = -2.733$, $p < .01$), compared to controls (M = 1.13, SD = 0.21), and additionally, larger right amygdala volumes (M = 1.34, SD = 0.29), relative to controls (M = 1.14, SD = 0.20; $t(54) = -2.997$, $p < .01$; see Table 5).

There was also a significant interaction between group, age group, social anxiety in predicting left ($F(4, 72) = 5.58$, $p < .01$) and right amygdala volumes ($F(4, 72) = 3.80$, $p < .01$). A bivariate correlational analysis was generated again to assess the relationships between anxiety and amygdala volume in each age group. In younger individuals with ASD, there was a significant positive correlation between social anxiety scores and left amygdala volume (r = .583, $p < .05$; see Figure 5). There were no significant relationships between social anxiety and amygdala volume in older individuals with ASD, however, there was a positive correlation between social anxiety and age (r = .588, $p < .05$; see Figure 6). No significant correlations were evident in controls of any age group.

Medication

While comparing mean amygdala volumes between medicated and non-medicated participants, those on medication displayed significantly larger left ($t(86) = -2.750$, $p < .01$) and right amygdala volumes ($t(86) = -2.189$, $p < .05$). However, when comparing medicated and non-medicated participants' volumes within the ASD group, there were no significant differences. Medication significantly interacted in the relationship between group and social anxiety ($F(1, 77) = 14.848$, $p < .01$), as well as separation anxiety ($F(1, 77) = 15.459$, $p < .01$). Medicated participants had significantly higher levels of social anxiety, relative to non-medicated participants ($t(73) = -2.158$, $p < .05$). Within the ASD group, medicated participants displayed significantly higher levels of social anxiety ($t(26) = -2.250$, $p < .05$) and separation anxiety ($t(73) = -2.371$, $p < .05$).

To investigate whether the presence of medicated participants explained the results of this study, those taking medication were excluded and the previous analyses were run. Because there were no controls on medication, findings exclusively within the control group were unaffected. Group X social anxiety X amygdala volume interactions, as well as group X age group X social anxiety X amygdala volume interactions, were no longer significant. However, Significant differences in left ($t(72) = -2.647$, $p < .05$) and right amygdala volume ($t(72) = -2.442$, $p < .05$), between the ASD group and controls remained. In the ASD group, left amygdala volume still positively correlated with social anxiety ($r = .487$, $p < .05$), and additionally with physical symptoms ($r = .563$, $p < .05$). There was also a positive correlation between right amygdala volume and social anxiety ($r = .585$, $p < .05$), and physical symptoms ($r = .633$, $p < .05$) as well.

Discussion

Main Findings

As predicted, participants with ASD displayed significantly larger bilateral amygdala volumes, as well as a significantly higher amount of anxiety. Left and right amygdala volumes positively correlated with social anxiety scores in the ASD group, while no significant relationships were evident in controls. These results were consistent with previous studies of ASD, finding increased amygdala volume in youth (Nordahl et al., 2012; Sparks et al., 2002; Buitelaar, Groen, Teluij, & Tendolkar, 2010), as well as increased anxiety (Ollendick, Oswald, Scahill, & White, 2009; Kuusikko et al., 2008; Kleinhans et al., 2010; Juranek et al., 2006). Juranek and colleagues (2006) also found a positive relationship between anxiety and right amygdala volume using the CBCL; although a limitation this method is that it does not exclusively measure anxiety, nor does it signify the type of anxiety measured. An advantage to using MASC is that the survey solely measures symptoms of pediatric anxiety, as well as specific anxiety traits. Because of this, I was able to identify heightened social anxiety as a predictor of increased bilateral amygdala volume in ASD. To the best of my knowledge, this study is the first to identify this relationship.

Furthermore, this study hypothesized that group differences in amygdala volume would interact with age group, in that differences in volume between ASD and control groups would only remain significant in younger participants. Contrary to these expectations, older participants displayed an even larger discrepancy in amygdala volumes. A possible explanation as to why these results differ from that of Schumann and colleagues (2004) may rely on the relatively smaller number of participants with ASD. When those with ASD were split into age groups, there were only 15 individuals younger than 13 years and 18 individuals older than 13 years. Because of these smaller group sizes, it is possible that participants with ASD in each age group were not an accurate representation of the ASD youth population.

Limitations

Additionally, many participants with ASD were excluded due to verbal and nonverbal IQ scores < 85. Because Schumann and colleagues (2004) included a wide distribution of ASD diagnosis types, their study included 19 individuals with IQ scores < 70. My exclusion of participants with lower functioning autism presents a possible limitation to this study. Participant ASD diagnoses include: Autistic disorder (AD), high-functioning autism (HFA), and pervasive-developmental disorder- not otherwise specified (PDD-NOS). The significant amount of participants with higher functional abilities may also lead to a larger amount of anxiety in the ASD sample. As suggested by Attwood (2000), individuals with higher functioning ASD and Asperger's syndrome may be significantly more anxious than those with lower functioning ASD, due to an increased awareness of their impairments during social interactions. Thus, future investigations focusing on social anxiety in ASD may benefit by recruiting from a diverse array of functional abilities.

When medicated participants were excluded, group differences in amygdala volume within younger participants were no longer significant. These results provide many implications on the role of psychotropic medication in amygdala volume-anxiety interactions in ASD. Findings of increased social anxiety in medicated participants are not surprising, as many medications were prescribed to alleviate anxiety symptoms (e.g., SSRIs, anxiolytics). It is also possible, perhaps, that amygdala volume did not decrease in adolescents with ASD due to the inhibitory or excitatory effects their medications may hold on amygdala activity.

Hyperactivity of the amygdala has been observed individuals with ASD while viewing emotional faces (Dalton et al., 2005; Weng et al., 2011; Monk et al., 2010). Decreased amygdala habituation, the prolonged activation of the amygdala in response to facial stimuli, has also been

suggested (Kleinhans et al., 2009), and as of recently, found to correlate to the degree of social impairments observed in ASD (Carrasco, Lord, Monk, Swartz, & Wiggins, 2013). In line with these observations, amygdala hyperactivity has been proposed as an indication of aversive reactions to emotional faces, evoking an increased emotional response (Dalton, et al., 2005; Chilvers, Corden, & Skuse, 2007). As speculated by Buitelaar, Groen, Teluij, and Tendolkar (2010), stress and anxiety in children with ASD may lead to increased activity in the amygdala. In turn, this functional increase may eventually lead to excitotoxic effects as they age into adulthood. Post-mortem findings of unusually small and densely packed amygdala neurons in ASD further support this theory (Bauman & Kemper, 1993). Still, the exact mechanism of these abnormalities remains unknown.

Finally, an assessment of accuracy of child-reported anxiety measures suggests another possible limitation to this study. Maddox, Schry, and White (2012) found that adolescents using MASC tended to significantly underreport their anxiety levels in comparison to parent-reported MASC and clinical analyses. Future studies measuring anxiety in ASD may receive more accurate data from utilizing both child and parent-reporting techniques.

Conclusions

The results of this study suggest that within ASD, larger amygdala volume is associated with a higher degree of social anxiety. This relationship was also influenced by age. Further investigations relating these interactions to particular ASD diagnoses, as well as additional measures of anxiety, social behavior, and amygdala activity may help distinguish possible underlying mechanisms of ASD impairments.

References

Achenbach, T. M. (1991). Integrative guide for the 1991 CBCL/4−18, YSR, and TRF Profiles. Burlington, VT: University of Vermont Department of Psychiatry.

American Psychological Association. (2000).

Attwood, T. (2000). Strategies for improving the social integration of children with Asperger syndrome. *SAGE Publications,* 4(1), 85-100.

Aylward, E.H., Minshew, N.J., Goldstein, G., Honeycutt, N.A., Augustine, A.M., Yates, K.O., Barta, P.E., & Pearlson, G.D. (1999). MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology*, *53*, 2145-2150.

Baron-Cohen, S., Ring, H.A., Bullmore, E.T., Wheelwright, S., Ashwin, C., Williams, S.C.R. (2000). The amygdala theory of autism. *Neuroscience and Behavioral Reviews, 24*(3), 355-364.

Bellini, S. (2004). Social skill deficits and anxiety in high-functioning adolescents with autism spectrum disorders. *Focus on Autism and Other Developmental Disabilities*, *19*(2), 78-86.

Corbett, B.A., Carmean, V., Ravizza, S., Wendelken, C., Henry, M.L., Carter, C., & Rivera, S.M. (2008). A functional and structural study of emotion and face processing in children with autism. *Psychiatry Research: Neuroimaging*, *173*, 196-205.

Corden, B., Chilvers, R., & Skuse, D. (2007). Avoidance of emotionally arousing stimuli predicts social-perceptual impairment in Asperger's syndrome. *Neuropsychologia*, *46*, 137-147.

Courchesne, E., Karns, C.M., Davis, H.R., Ziccardi, R., Carper, R.A., Tigue, Z.D., Chisum, H.J., Moses, P., Pierce, K., Lord, C., Lincoln, A.J., Pizzo, S., Schreibman, L., Haas, R.H.,

Askhoomoff, N.A., & Courchesne, R.Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology*, *57*(2), 245-254.

Dalton, K.M., Nacewicz, B.M., Johnstone, T., Schaefer, H.S., Gernsbacher, M.A., Goldsmith, H.H., Alexander, A.L. & Davidson, R.J. (2005) Gaze fixation and the neural circuitry of face processing in autism. *Nature Neuroscience*, *8*(4), 519-526.

Groen, W. Teluij, M., Buitelaar, J., & Tendolkar, I. (2010). Amygdala and hippocampal enlargement during adolescence in autism. *Journal of the American Academy of Child & Adolescent Psychiatry*, *49*(6), 552-560.

Howard, M.A., Cowell, P.E., Boucher, J., Broks, P., Mayes, A., Farrant, A., & Roberts, N. (2000). Convergent neuroanatomical and behavioral evidence of an amygdala hypothesis of autism. *NeuroReport, 11*(13), 2931-2935.

Juranek, J., Filipek, P.A., Berenji, G.R., Modahl, C., Osann, K., & Spence, M.A. (2006). Association between amygdala volume and anxiety level: magnetic resonance imaging (MRI) study in autistic children. *Journal of Child Neurology*, *21*(12), 1051-1058.

Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child, 2*, 217-250.

Kemper, T. L. & Bauman, M. L. (1993). The contribution of neuropathologic studies to the understanding of autism. *Neurologic Clinics, 11*, 175-187.

Kleinhans, N.M., Johnson, L.C., Richards, T., Mahurin, R., Greenson, J., Dawson, G., Aylward, E. (2009). Reduced neural habituation in the amygdala and social impairments in autism spectrum disorders. *American Journal of Psychiatry*, 166, 467-475.

Kleinhans, N.M., Richards, T., Weaver, K., Johnson, L.C., Greenson, J., Dawson, G., Aylward, E. (2010). Association between amygdala response to emotional faces and social anxiety in autism spectrum disorders. *Neuropsychologia, 48*, 3665-3670.

Kuusikko, S., Pollock-Wurman, R., Jussila, K., Carter, A. S., Mattila, M. L., Ebeling, H., Pauls, D.L., & Moilanen, I. (2008). Social anxiety in high-functioning children and adolescents with autism and Asperger syndrome. *Journal of Autism and Developmental Disorders*, *38*, 1697–1709.

Lord, C. Risi, S., Lambrecht, L., Cook, E.H. Jr., Leventhal, B.L., DiLavore, P.C., Pickles, A., & Rutter, M. (2000). The autism diagnostic observation schedule-generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders*, *30*, 205-223.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders*, *24*, 659-685.

MacMaster, F.P., Mirza, Y. Szeszko, P.R., Kmiecik, L.E., Easter, P.C., Preeya, S., … & Rosenberg, D.R. (2008). Amygdala and hippocampal volumes in familial early onset major depressive disorder. *Biological Psychiatry, 63,* 385-390.

March, J., Parker, J., Sullivan, K., Stallings, P. & Conners, C. (1997). The Multidimensional Anxiety Scale for Children (MASC): Factor structure, reliability and validity. *Journal of the American Academy of Child & Adolescent Psychiatry*, *36*(4), 554-565.

Monk, C.S., Weng, S.J., Wiggins, J.L., Kurapati, N., Louro, H.M.C, Carrasco, M., Maslowsky, J., Risi, S., Lord, C. (2010). Neural Circuitry of emotional face processing in autism spectrum disorders. *Journal of Psychiatry and Neuroscience, 35*(2), 105-114.

Nacewicz, B.M., Dalton, K.M., Johnstone, T., Long, M.T., McAuliff, E.M., Oakes, T.R., Alexander, A.L., & Davidson, R.J. (2006). Amygdala volume and nonverbal social impairment in adolescent and adult males with autism. *Archives of General Psychiatry,*

63, 1417-1428.

Nordahl, C.W., Scholz, R., Yang, X., Buonocore, M.H., Simon, T., Rogers, S., & Amaral, D.G. (2012). Increased rate of amygdala growth in children aged 2 to 4 years with autism spectrum disorders: a longitudinal study. *Archives of General Psychiatry, 69*(1), 53-61.

Schumann, C.M., Hamstra, J., Goodlin-Jones, B.L., Lotspeich, L.J., Kwon, H., Buonocore, M.H., Lammers, C.R., Reiss, A.L., & Amaral, D.G. (2004). The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *Journal of Neuroscience*, *24*, 6392-6401.

Sparks, B.F., Friedman, S.D., Shaw, D.W., Aylward, E.H., Echelard, D., Artru, A.A., Maravilla, K.R., Giedd, J.N., Munson, J., Dawson, G., & Dager, S.R. (2002). Brain structural abnormalities in young children with autism spectrum disorder. *Neurology, 59,* 184-192.

Swartz, J.R., Wiggins, J.L., Carrasco, M., Lord, C., & Monk, C.S. (2013). Amygdala habituation and prefrontal functional connectivity in you with autism spectrum disorders. *Journal of the American Academy of Child & Adolescent Psychiatry, 52*(1), 84-93.

Weng, S.J., Carrasco, M., Swartz, J.R., Wiggins, J.L., Kurapati, N., Liberzon, I., Risi, S., Lord, C., & Monk, C.S. (2011). Neural activation to emotional faces in adolescents with autism spectrum disorders. *Journal of Child Psychology and Psychiatry, 52*(3), 296-305.

White, S.W., Oswald, D., Ollendick, T., & Scahill, L. (2009). Anxiety in children and adolescents with autism spectrum disorders. *Clinical Psychology Review*, *29*, 216-229.

White, S. W., Schry, A. R., & Maddox, B. B. (2012). Brief report: The assessment of anxiety in high-functioning adolescents with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 42*(6), 1138-1145.

Author Note

Sarah L. Alberti, Department of Psychology, University of Michigan.

First, I would like to thank Dr. Christopher Monk and Ms. Johnna Swartz for providing me with support, feedback, and the opportunity to work collaboratively with them. Thanks to Ms. Swartz, as well as Dr. David Rosenberg and Mr. Philip Easter of Wayne State University, I was able to learn the manual tracing skills necessary to collect and analyze the data of this study. Finally, I thank Dr. Catherine Lord, the University of Michigan Autism and Communications Disorders Center, as well as Ms. Jillian Lee Wiggins, Mr. Daniel Smith, and all members of the Translational and Developmental Neuroscience Lab for aiding me in the execution of this thesis.

This thesis was conducted through Psychology courses 424 and 426 Section 586 at the University of Michigan.

Correspondence concerning this article should be addressed to Sarah L. Alberti, University of Michigan, 1130 Hill Street, Ann Arbor, MI, 48104.

E-mail: alberti@umich.edu

Table 1.

Participant Characteristics

	ASD		Control		
Measure	*n*	Mean (*SD*)	*n*	Mean (*SD*)	Group differences
Age (years)	33	13.53 (2.29)	54	14.59 (2.95)	$t(85) = 1.78, p > .05$
Verbal IQ	33	114.21 (20.82)	55	113.80 (13.48)	$t(86) = -.11, p > .05$
Nonverbal IQ	32	107.56 (19.28)	52	103.04 (12.13)	$t(82) = -1.32, p > .05$
Physical symptoms	28	8.96 (7.02)	50	6.38 (4.54)	$t(76) = -1.97, p > .05$
Social anxiety	28	11.18 (6.46)	50	7.50 (4.60)	$t(76) = -2.92, p < .01$*
Harm avoidance	28	13.21 (5.36)	50	11.56 (4.73)	$t(76) = -1.41, p > .05$
Separation anxiety	28	8.36 (4.6)	50	5.52 (3.54)	$t(76) = -3.04, p < .01$*
LA volume (cm^3)	33	1.30 (.27)	55	1.12 (.19)	$t(86) = -3.58, p < .01$*
RA volume (cm^3)	33	1.32 (.26)	55	1.16 (.22)	$t(86) = -3.23, p < .01$*

Note. LA = left amygdala; RA = right amygdala. Physical symptoms, social anxiety, harm avoidance, and separation anxiety are subscales of the Multidimensional Anxiety Scale for Children (MASC; March et al., 1997). Age was missing for one control. Nonverbal IQ was missing for one participant with ASD and three controls. Five participants with ASD and five controls were missing MASC scores.

* Difference is significant.

Table 2.

Medication

	ASD		Control		Total	
Medication type	*n*	%	*n*	%	*n*	%
Any	14	42.4	NA	NA	14	15.9
SSRI	3	9.1			3	3.4
Stimulant, ADHD	8	24.2			8	9.1
Non-stimulant, ADHD	3	9.1			3	3.4
Anxiolytic	2	6.1			2	2.3
Antipsychotic	3	9.1			3	3.4
Combination	4	12.1			4	4.5

Note. SSRI = selective serotonin reuptake inhibitor; ADHD = attention deficit hyperactivity disorder; NA = not applicable. No controls were currently taking medication at the time of scan.

Table 3.

Interactions in Predicting Left and Right Amygdala Volume

	Group * social anxiety		Group * age group * social anxiety	
Dependent variable	R^2	F (2, 75)	R^2	F (4, 72)
Left amygdala volume	.232	11.338**	.194	5.582**
Right amygdala volume	.158	7.038**	.128	3.787**

Note. There was a significant group X social anxiety interaction, as well as a group X age group X social anxiety interaction in predicting bilateral amygdala volumes.

**Difference is significant at $p < .01$

Table 4.

Bivariate Correlations: Amygdala Volume, Anxiety, and Age Between Groups

Measure	ASD		Control	
	LA	RA	LA	RA
Physical Symptoms	.364	.373	-.115	-.110
Social anxiety	.455*	.402*	.021	-.186
Harm avoidance	.345	.324	-.170	-.229
Separation anxiety	.334	.256	-.104	-.216
Age	.173	.204	.181	.080

Note. LA = left amygdala; RA = right amygdala.

Participants with ASD displayed positive correlations between social anxiety and left amygdala volume, as well as right amygdala volume.

*Difference is significant at $p < .05$

Table 5.

Group Differences in Amygdala Volume (cm^3), Divided by Age

	Ages < 13 (years)			Ages > 13 (years)		
	ASD (n = 15)	Control (n = 38)		ASD (n = 18)	Control (n = 38)	
Measure	*M* (*SD*)	*M* (*SD*)	*t*(29)	*M* (*SD*)	*M* (*SD*)	*t*(54)
Left amygdala	1.30 (.31)	1.10 (.16)	-2.27*	1.30 (.24)	1.13 (.21)	-2.73**
Right amygdala	1.30 (.22)	1.19 (.26)	-1.32	1.34 (.29)	1.14 (.20)	-3.00**

Note. When participants were divided into age groups, younger participants displayed a significant difference in left amygdala volume between ASD and control groups. This difference did not remain significant in the right hemisphere. Moreover, participants older than 13 years displayed a significant difference in both left and right amygdala volumes between ASD and control groups.

*Difference is significant at $p < .05$

**Difference is significant at $p < .01$

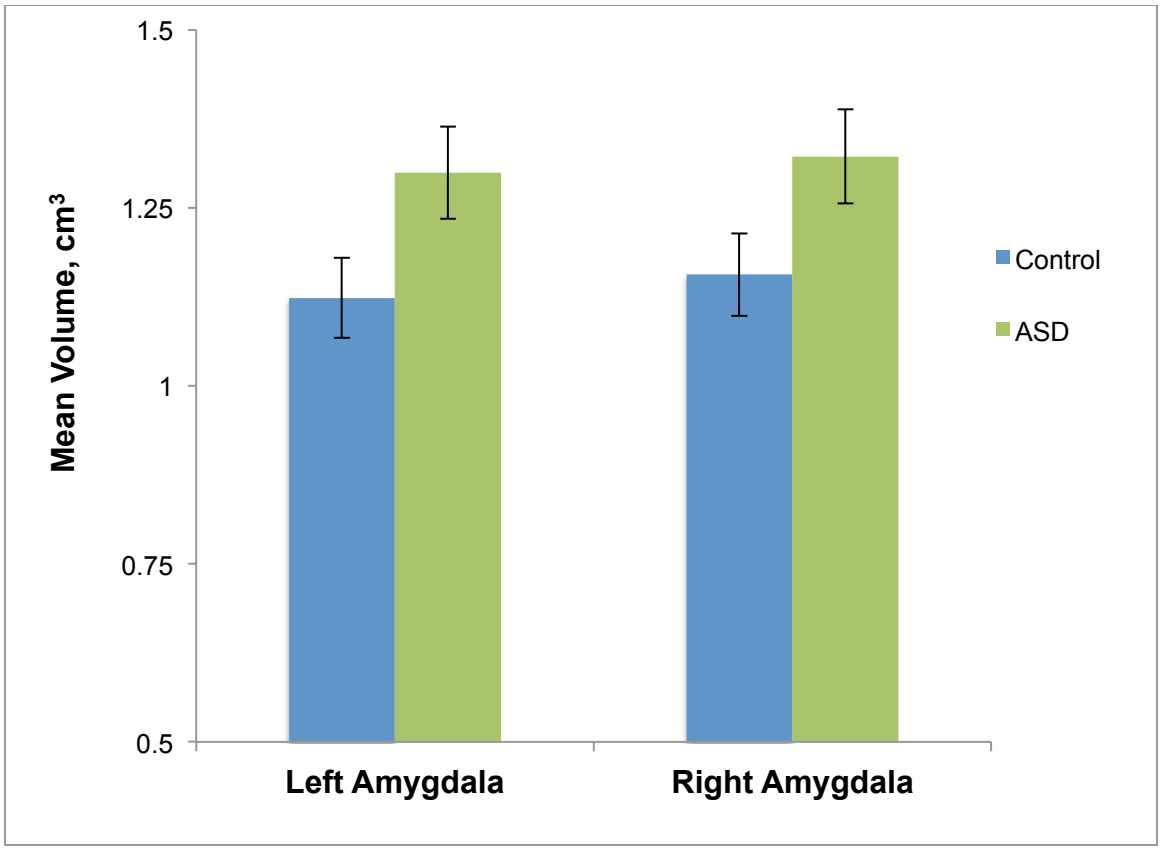


Figure 1. Group differences in amygdala volume. This bar graph illustrates the significantly lager mean volumes in the ASD group for both the left and right amygdala. Standard errors are represented by the vertical error bars on each column.

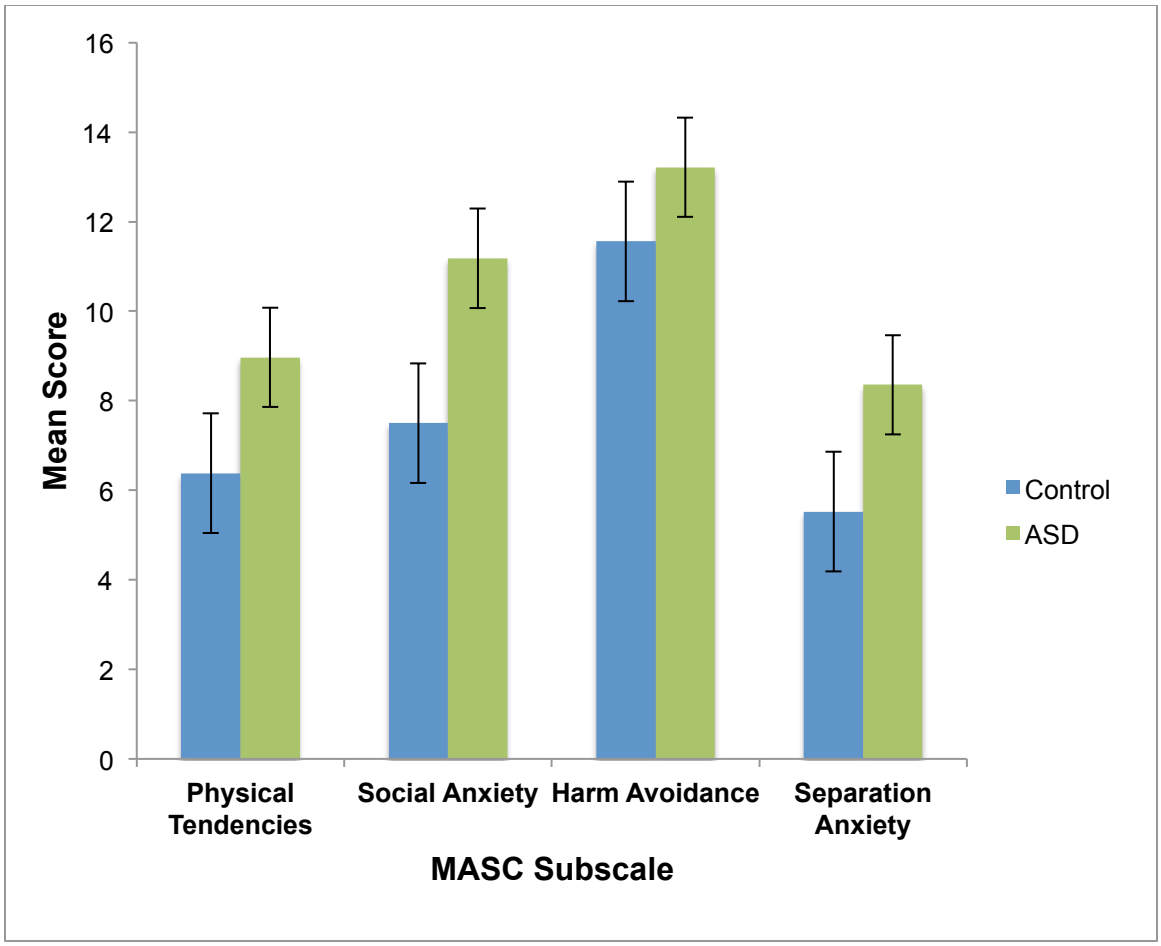


Figure 2. Group differences in anxiety. Although the ASD group had higher mean scores in every anxiety category, these group differences were only significant between social anxiety and separation anxiety scores. Standard errors are represented by the vertical error bars on each column.

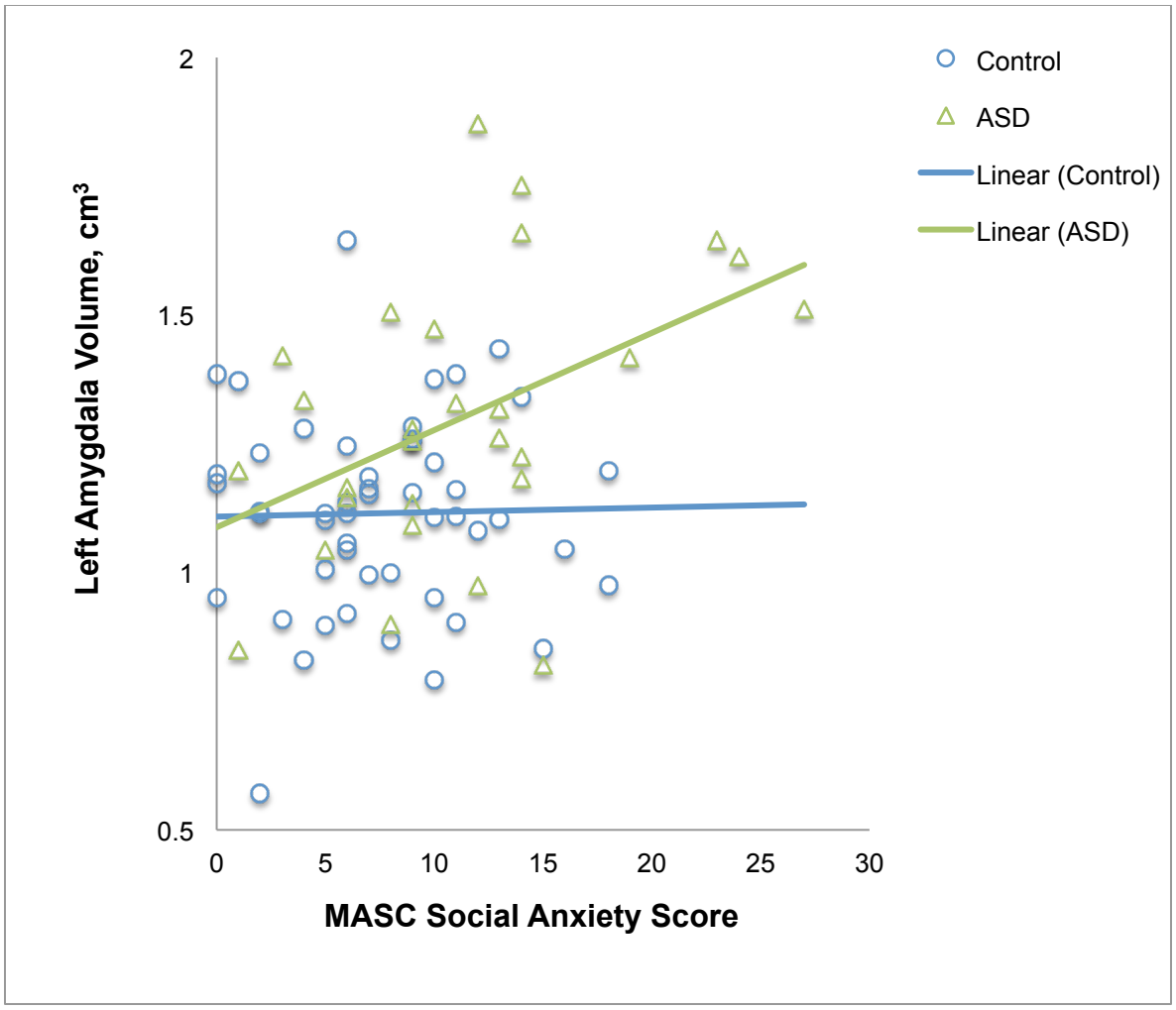


Figure 3. Left amygdala volume (cm^3) and social anxiety. This scatter plot compares the relationships between left amygdala volume and MASC social anxiety sub scores. No significant relationship was evident in controls, but a positive relationship was evident in the ASD group.

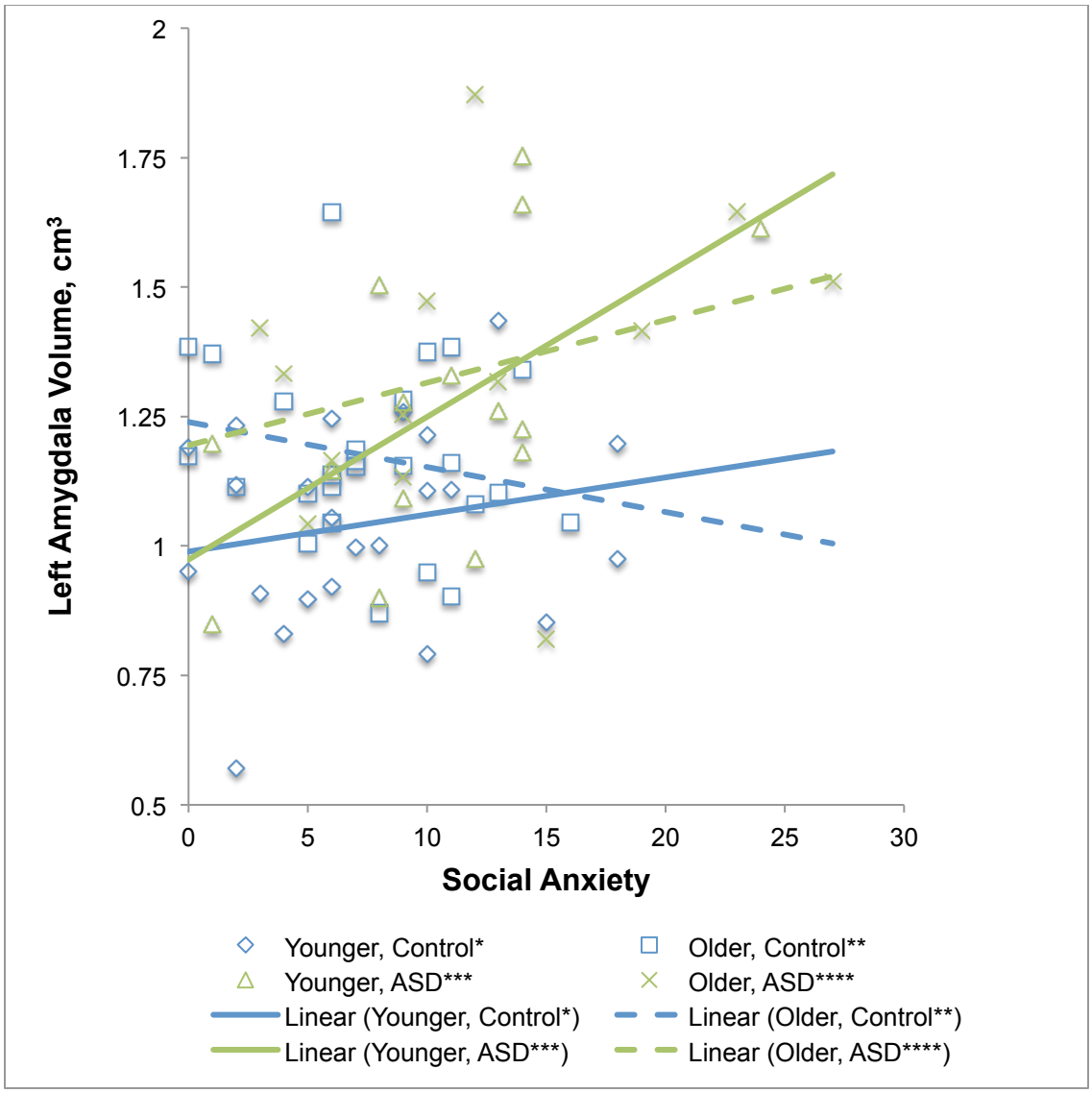


Figure 5. Age group differences in the relationship between social anxiety and left amygdala volume (cm^3). In younger participants with ASD, social anxiety correlated positively with left amygdala volume. There were no significant relationships between social anxiety and volume in older participants with ASD, nor were there in controls of any age group.

*$R^2 = .064$

**$R^2 = .009$

***$R^2 = .343$

****$R^2 = .113$

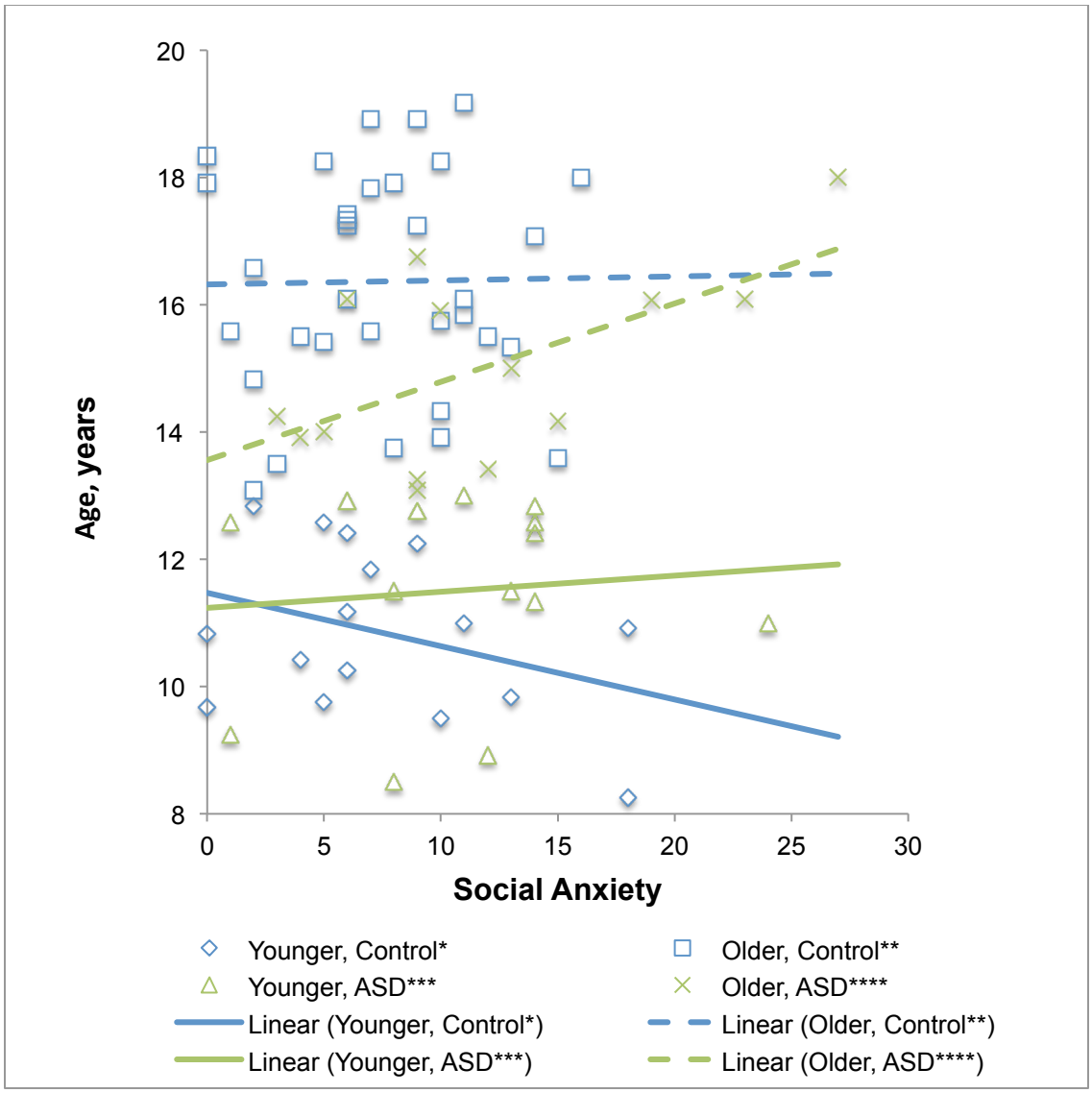


Figure 6. Age group differences in the relationship between social anxiety and age. In older participants with ASD, social anxiety correlated positively with age. There were no significant relationships between social anxiety and age in younger participants with ASD, nor were there in controls of any age group.

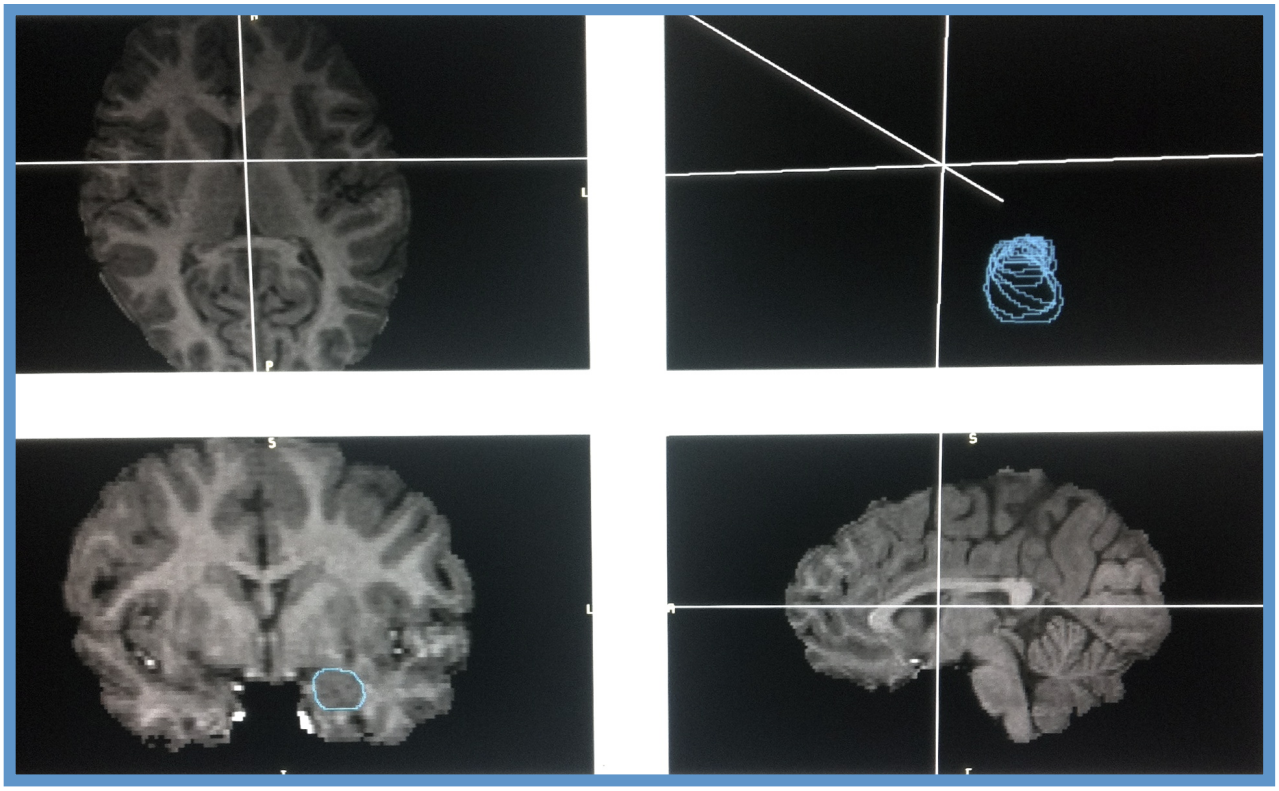
*$R^2 = .124$

**$R^2 < .001$

***$R^2 = .009$

****$R^2 = .346$

Appendix



A sample window of the MRI analyzing software BRAINS2, displaying a left amygdala tracing. Raters traced on a coronal plane, moving rostrally, as illustrated in the bottom left.