IMPLICATIONS OF AMYGDALA ABNORMALITIES

IN AUTISM SPECTRUM DISORDER

By

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Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by social and behavioral deficits. This disorder affects more than two percent of children in the United States and can vary in severity. Little is known about the pathology of ASD, however, it is clear that both genetic and environmental factors contribute to the development of the disorder. Furthermore, it has been found that amygdala abnormalities may be part of the cause of the symptoms that result in a diagnosis ASD. Key abnormalities that have been investigated include increased amygdala volume, decreased habituation of the amygdala, and aberrant connections from the amygdala to cortical regions involved in social and repetitive behaviors. This literature review integrates findings from multiple imaging studies that look at the aforementioned amygdala abnormalities as well as studies that investigate typical developmental trajectories in an attempt to uncover a potential piece of the mechanisms underlying the development of ASD. A better understanding of these mechanisms will help in the development of safe and effective treatments for individuals with ASD.

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and interaction and repetitive patterns of behavior (American Psychiatric Association). Common accompanying symptoms include intellectual impairment, language impairment, and sensory overresponsivity (SOR) and severity of the disorder varies, ranging from mild to severe phenotypes. Symptoms present early in development and children can be diagnosed as early as age 2, however, diagnosis typically occurs at about age 4.

The prevalence of ASD in the United States is 1 in 41, or 2.43%, and it is steadily increasing (Xu et al. 2018). In 2008, the prevalence was 1 in 88 (1.14%) and in 2000, it was only 1 in 150 (0.67%). This striking increase may be due to many different factors, one being different diagnostic criteria in the newest edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM), the DSM-5, which was released in 2013. The diagnostic criteria for ASD changed to include autistic disorder (AD), Asperger's syndrome, and pervasive developmental disorder not otherwise specified (PDD-NOS). Additionally, the DSM-5 also allows for multiple diagnoses, which was not allowed by previous editions of the DSM. A second contributing factor may be the access to specialized services and special education that is given to children with an ASD diagnosis, which may cause doctors to diagnosis children who are on the cusp of clinical criteria (Wright 2017). However, there is likely some natural increase in occurrence of ASD due to genetic and environmental risk factors (Figure 1).

With this profound increase in prevalence over the last few decades, regardless of the reason, it has become enormously important to understand the underlying brain changes in order to maximize therapeutic efforts directed toward these children. By the age of 4, roughly the age

that most children are diagnosed, a substantial portion of critical windows of development in which developmental processes proceed optimally have passed. This may be especially important in development of the "social brain" circuitry, which is foundational for many aspects of subsequent development, including relationships and ability to function in school.

Because of the social impairments associated with ASD, it has long been thought that it is caused in part in amygdala abnormalities. The amygdala is considered part of the "social brain", first described by L. Brothers in 1990, along with the orbito-frontal cortex (OFC) and superior temporal sulcus and gyrus. In 1999, Baron-Cohen et al. developed the amygdala theory of autism, which states that abnormalities in the amygdala are a key component in the pathology of autism. The abnormalities described in the theory of autism include abnormal size of the amygdala and aberrant activation.

Participants with ASD from all of the following studies measuring amygdala abnormalities were previously diagnosed and, with the exception of the Gibbard et al. 2018, had diagnoses confirmed by clinician using the Autism Diagnostic Interview-Revised (ADI-R), the Autism Diagnostic Observation Schedule (ADOS), and the most recent version of the DSM that was available at the time (DSM-IV or DSM-5).

Amygdala Structure and Function

Typical functions of the amygdala include drive-related behavior, emotional regulation, and social behavior. The amygdala develops from neurons that originate in the diencephalic vesicle and then colonize in the telenceohalic amygdaloid complex during embryonic development (García-Moreno et al. 2010). The amygdala itself does not differentiate into different nuclei and subregions until after birth. It comprises 13 nuclei, clustered into three

different groups: centromedial (CM), laterobasal (LB), and superficial (SF) (Figure 2). The LB region facilitates associative learning, the CM region has a role in generating behavioral responses, and the SF region is involved in olfactory and affective processes (LeDoux 2003; Heimer and Van Hoesan 2006; Gonzalez-Lima and Scheich 1986). It has also been found that the LB subregion is involved in processing of auditory input, and that auditory stimulation causes the neurons in the LB and CM regions to oscillate. A disturbance in this oscillation pattern due to an abnormality could result in abnormal stimulation of the amygdala in either direction, i.e., overstimulation or increased inhibition. Additionally, it was found that the CM lateralization patterns differed from the patterns of the LB and SF subregions (Ball et al. 2007).

Amygdala Size Increased in ASD

Using magnetic resonance imaging (MRI) of 4-year-olds, Sparks et al. (2002) showed that amygdala size in individuals with ASD is increased bilaterally but proportionally to overall cerebral increase, which included both gray and white matter regions, but not cerebrospinal fluid or regions of non-brain matter. After controlling for age, Sparks et al. also showed that the boys with ASD showed significantly larger amygdala, while there was no significant difference found in amygdala size in girls with ASD, implying a sex difference (Figure 3). However, this could be due to the small number of female children included in the study compared to males. Like many other studies conducted before the release of the DSM-5, the Sparks et al. ASD group was subdivided into children diagnosed with AD and PDD-NOS. Consequently, this may have resulted in children being included in the study that may not have met the current criteria for ASD.

Other studies also have shown enlarged amygdalae in individuals with ASD. Most recently, Gibbard et al. (2018) used MRI scans to show that young adults (mean age 24 years) had increased amygdala volume. Kim et al. (2010) used MRI to show bilateral enlargement of amygdala in children with ASD (ages 6 to 7 years) compared to age-matched typical developing (TD) children, and specifically showed enlargement of the LB subregion, which, as noted earlier, is important in processing auditory input. Furthermore, this enlargement correlated with severity of social and communication symptoms (Figure 4). Similar to Sparks et al., Kim's group further separated the ASD group into AD and PDD-NOS.

Shen et al. (2016) used functional magnetic resonance imaging (fMRI) and concluded that children with ASD aged 3.5 years had volumetric increases in the right amygdala, but not the left, compared to age-matched TD children. Though this is not the common finding, it is interesting when considering that the left and right amygdala may have different functions in emotional regulation. It has been suggested that the right amygdala is involved in a dynamic emotional stimulus detection system, and the left amygdala is specialized for sustained stimulus evaluations. Furthermore, it has been suggested that the left amygdala is more active when presented with fearful versus happy stimuli (Wright et al. 2001). If this difference persists in further studies, it could explain deficits in social and emotional intelligence in fearful or negative situations.

Decreased Habituation in ASD

Decreased habituation has also been observed in individuals with ASD. Habituation refers to the diminishing of the activity or response of a brain structure to a frequently repeated or constant stimulus. Decreased habituation of the amygdala could result in inability to extract,

interpret and appropriately react to social cues from the environment, an impairment that is one of the key symptoms of ASD. Kleinhans et al. (2009) used MRI and a neutral face-processing task to test neural habituation in the amygdala of adults aged 22-24 years. They found that amygdala habituation was decreased bilaterally in the ASD group compared to the TD group (Figure 5). Swartz et al. (2013) used face-processing tasks and fMRI to test neural habituation in the amygdala in adolescents (mean age ~14 years). In this study, the participants were presented with fearful, happy, sad, and neutral faces. Amygdala habituation was again bilaterally decreased in the ASD group compared to controls, but only when presented with sad and neutral faces (Figure 6). Both studies showed correlations between the level of habituation and severity of symptoms in the ASD group.

Green et al. (2015) exposed participants to mildly aversive stimuli to test habituation, among other factors, in children with ASD compared to TD adolescents (mean age ~14 years). They also found decreased habituation in amygdala in youths with ASD with SOR but not the subgroup without SOR or the age-matched controls. Additionally, it was shown that the ASD group had stronger neural responses compared to the control group.

Aberrant Amygdala Connectivity in ASD

Many studies have also measured functional and structural connectivity of the amygdala to other regions of the brain in individuals with ASD. Green et al. (2015) looked at functional connectivity, using fMRI, between the amygdala and the OFC, which is, as previously stated, another brain region that is part of the "social brain". Functional connectivity examined whether the OFC showed an increase (positive connectivity) or decrease (negative connectivity) in activity as a function of increased activity of the amygdala, compared to the baseline for each

individual. TD adolescents showed positive connectivity between the right amygdala and the left OFC, however, ASD adolescents showed negative connectivity between the same regions. Within the ASD group, ASD without SOR showed greater negative connectivity than the ASD with SOR group, suggesting that the connectivity differences correlate with symptom severity (Figure 7).

Swartz et al. (2013) showed that when the participants were presented with sad faces, they observed decreased connectivity compared to TD individuals between the amygdala and the ventromedial prefrontal cortex (vmPFC), inputs from which regulate amygdala activity.

Gibbard et al. (2018) used diffusion tensor imaging (DTI) to visualize and measure white matter (WM) tracts from the amygdala to the cortex. The two measures they used were fractional anisotropy (FA), a measure of directional restriction of water diffusion, and mean diffusivity (MD), a measure of overall magnitude of diffusion. Because axons in WM tracts are aligned, water diffuses easily in parallel to the WM tracts, but not radically. Therefore, well-developed, large tracts would show greater FA. The study showed significantly increased right hemisphere amygdala-cortical connectivity in the ASD group compared to controls when measuring WM tracts using MD but not FA, but no significant differences in the left hemisphere MD or FA of amygdala-cortical tracts. Additionally, symptom severity in the ASD group was negatively correlated with both the level WM tract MD and FA separately (Figure 8).

Shen et al. (2016) used fMRI to show that the ASD group had weaker connectivity between the amygdala and medial prefrontal cortex (mPFC), bilateral temporal lobe, striatum, thalamus, cingulate cortex, and cerebellum, all of which are brain regions involved in social communication and repetitive behaviors (Figure 9). This study included only boys, mean age 3.5 years. The weakened connectivity correlated with increased ASD symptom severity.

Kleinhans et al. (2016) used fMRI to study subregional differences in amygdala functional connectivity with other brain regions. After analyzing data from adolescents and adults with ASD, aged 14-45, and TD controls, they found that there is a decrease in connectivity from the LB region of amygdala, and an increase in connectivity from the CM subregion and the SF subregion. As stated before, the LB subregion is involved in associative learning, the CM subregion is involved in generating behavioral responses, and the SF subregion is involved in olfactory and affective processes. Symptom severity of the ASD group was associated with the reduced connectivity from the LB subregion.

Conclusion

In conclusion, amygdala size is increased in patients with ASD, which provides further evidence for the amygdala theory of autism. Due to the wide span of ages in cross-sectional studies that have been conducted, it seems that the enlargement of the amygdala persists throughout development and adolescence and into adulthood. The enlargement observed is typically bilateral, but there have been reports of unilateral enlargement. Further studies that measure the functions of the right and left amygdala in relation to ASD symptoms will be needed to interpret unilateral versus bilateral enlargement. Lastly, both functional and structural connectivity from the amygdala seem to be altered in individuals with ASD. Interestingly, there was no correlation observed between amygdala volume and connectivity differences in either the ASD or TD group (Shen et al. 2016). Due to the amygdala's typical function in emotional regulation and social cognition, it is reasonable to conclude that these abnormalities are a common characteristic of the pathology of ASD.

Future Considerations

Many studies have almost all, if not all, male participants. This is likely due to the much higher apparent prevalence of ASD in males compared to females, 3.63% and 1.25%, respectively (Xu et al. 2018). It would be interesting to see future studies that include more female participants, however, it would be expected that the connectivity abnormalities would be different in females with ASD compared to their male counterparts. This is because TD females have different connectivity patterns compared to TD males and TD females have been found to have superior social cognition skills. There appears to be an early separation in the developmental trajectories of girls compared to boys, with girls showing higher levels of interhemispheric connectivity and boys showing higher levels of intrahemispheric connectivity early in development (Ingalhalikar et al. 2014). Since TD girls have a higher level of social skills compared to TD boys, diagnosing a girl with ASD may be more difficult as a decreased level of social skill would not be as obvious in that a girl with autism may be functioning socially like a TD boy. This could potentially explain, in part, the lower prevalence of diagnosis of ASD in girls compared to boys. However, studying amygdala abnormalities in more females with ASD could give a more comprehensive explanation as to why there is such a striking difference in the prevalence of the disorder in males compared to females, and if the connectivity differences in TD individuals result in similar pattern in individuals with ASD.

It also would be interesting to see a longitudinal study that follows TD and ASD children to see how the amygdala changes over time. Though there are many different studies that look at the amygdala using participants from a wide range of ages, a study that uses the same parameters and participants at different stages would allow for insight as to whether the abnormalities observed in the amygdala and its connections in individuals with ASD persist over time or go

through structural and/or functional changes throughout the lifespan. If it is found that latter is true, then therapies for individuals with ASD would be different depending on the age of the individual at the onset of therapy and would potentially need to change over time as the amygdala undergoes change.

Learning more about the nature of amygdala abnormalities in individuals with ASD compared with TD individuals will be crucial in pinpointing the pathology and mechanism underlying ASD symptomology.

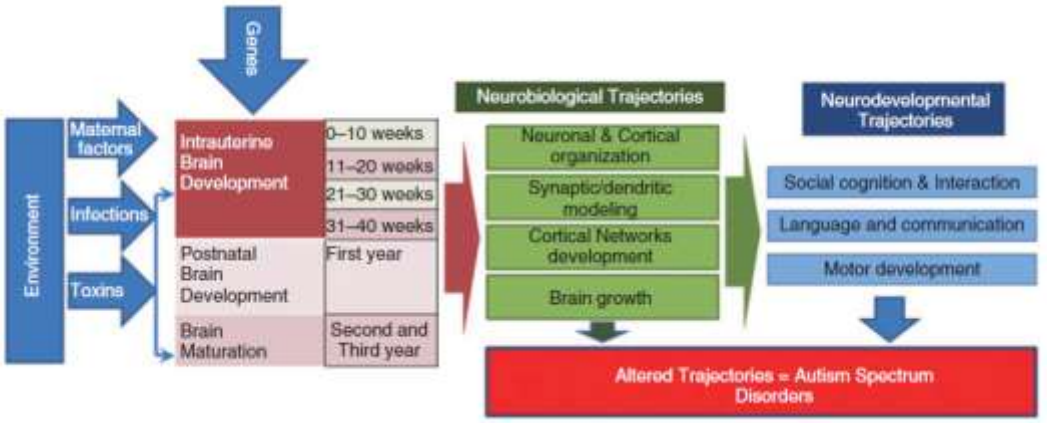

Figure 1: *ASD is likely caused by genetic and environmental factors that influence pre- and postnatal brain development (Pardo and Eberhart, 2007).*

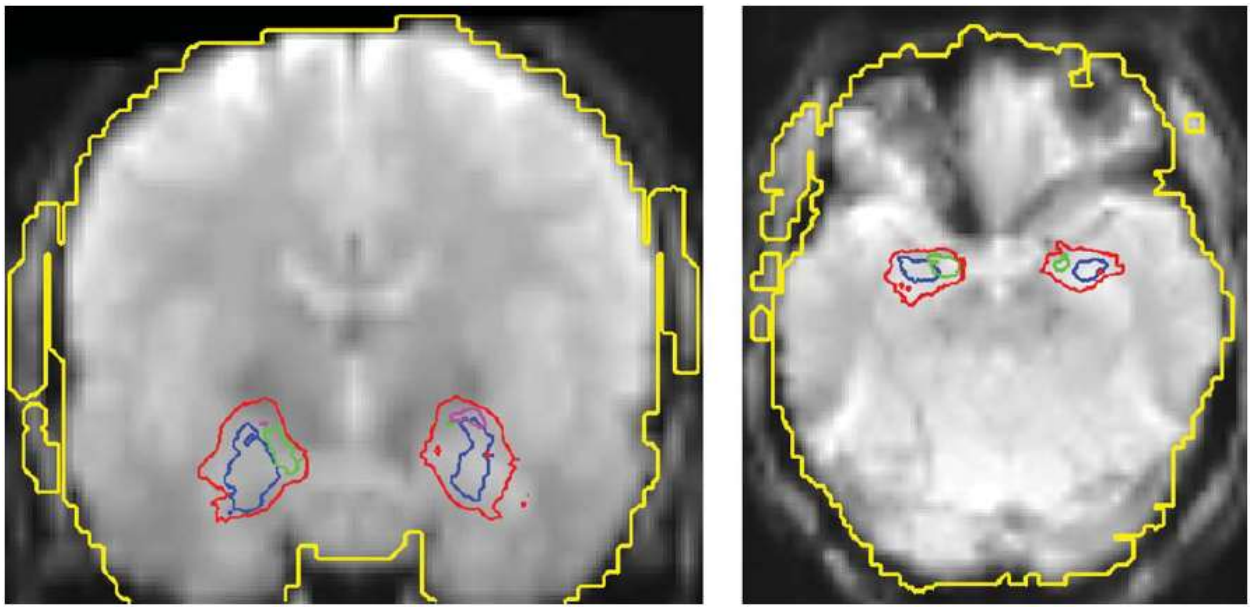

Figure 2: *fMRI scan with outlines of the amygdala and its subregions. The amygdala is outlined in red, the LB subregion in blue, the SF subregion in green, and the CM subregion in magenta.*

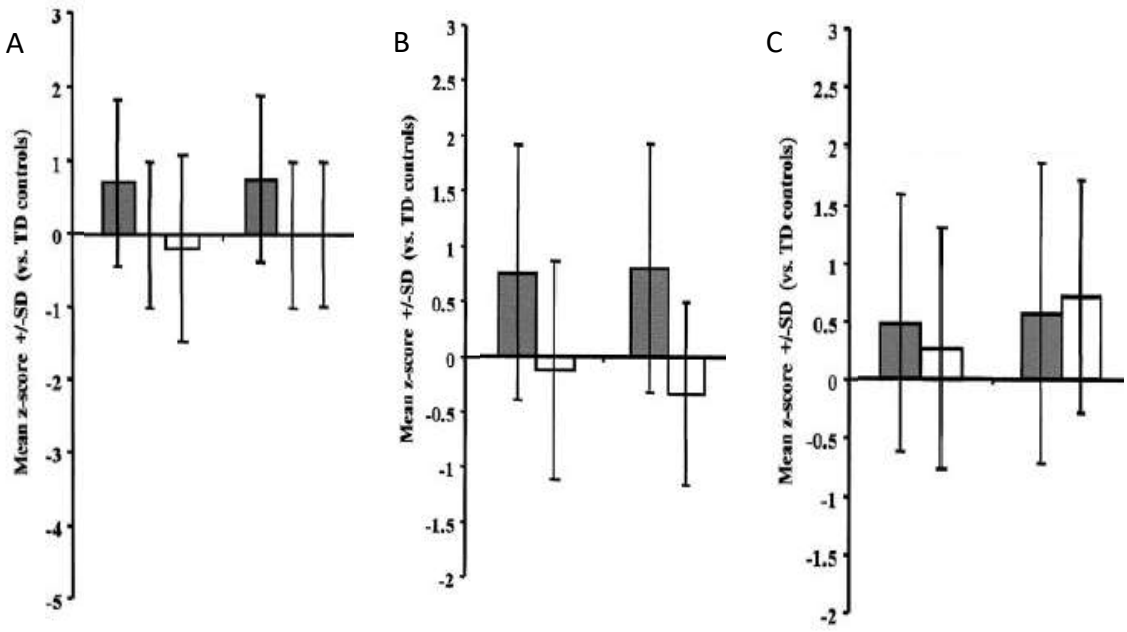

***Figure** 3: Quantification of bilateral amygdala volume increase in children (average age ~4 years) with ASD or development disorder (DD), normalized to typically developing (TD) children. (A) all children; grey bar is ASD, middle line in TD, and white bar is DD. (B) Boys only and (C) girls only; grey bar is ASD and white bar is DD, normalized to combined TD. Only significant increase in boys, but not combined or girls, and increase was proportional to overall cerebral increases (Figure adapted from Sparks et al. 2002).*

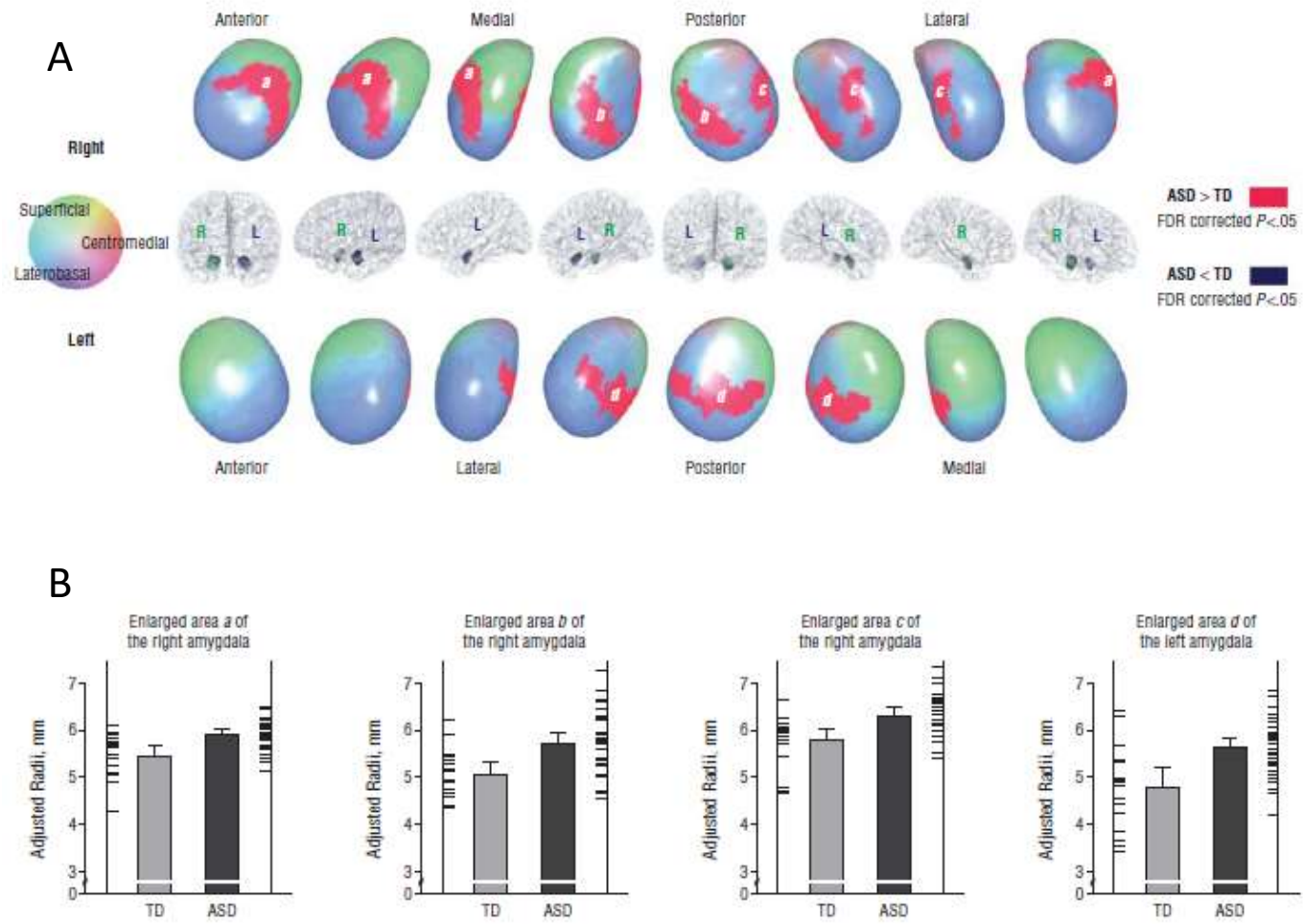

Figure 4: *(A) Enlarged amygdala areas in children ages 6-7 years with ASD (top) compared to age-matched TD children (bottom). Amygdala size increase was bilateral in ASD group. (B) Graphical representation of amygdala subregional increases in ASD children compared to TD children. Enlargement was in the laterobasal (LB) subregion of both right (regions a, b, and c) and left (region d) amygdala. Increases in LB amygdala subregion were correlated with phenotypic severity in the ASD group (Kim et al. 2010).*

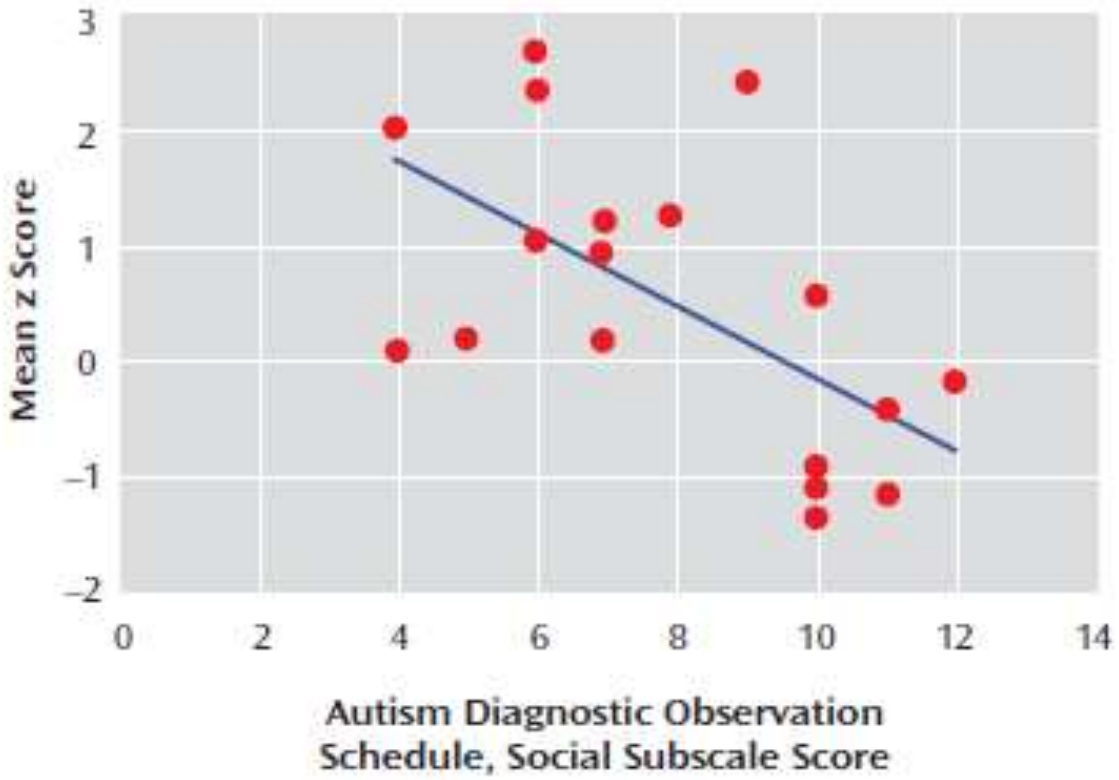

Figure 5: Decreased habituation to neutral faces in the right amygdala correlates with more severe symptoms in individuals with ASD (Kleinhans et al. 2009).

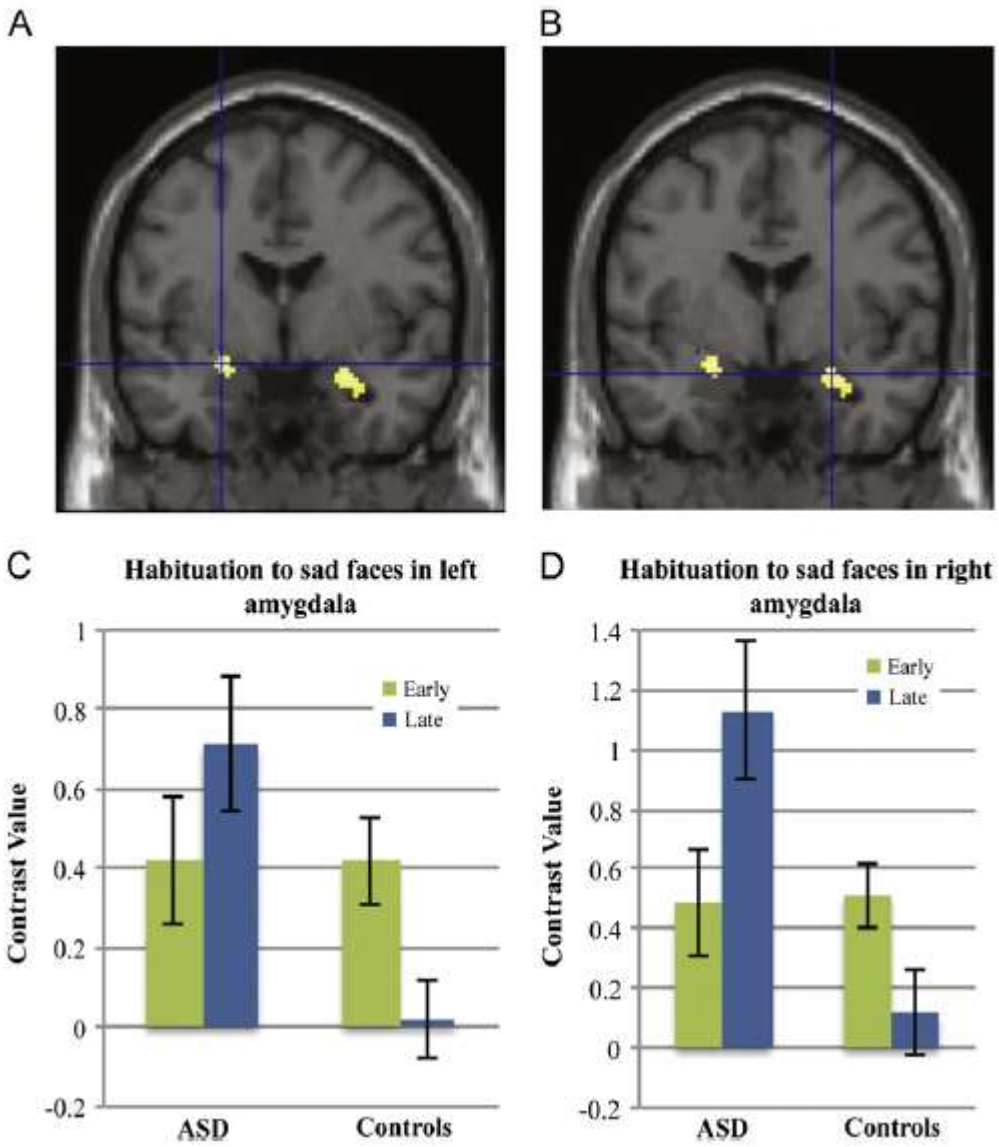

Figure 6: *ASD group shows significantly less habituation when presented with late sad faces compared to controls. (A and B) fMRI showing group difference of habituation to sad faces in left (A) and right amygdala (B) of controls compared to ASD group (mean age ~14 years). (C and D) Mean contrast values for early and late sad faces in ASD compared to controls in left (C) and right amygdala (D). ($p < 0.001$) (Swartz et al. 2013).*

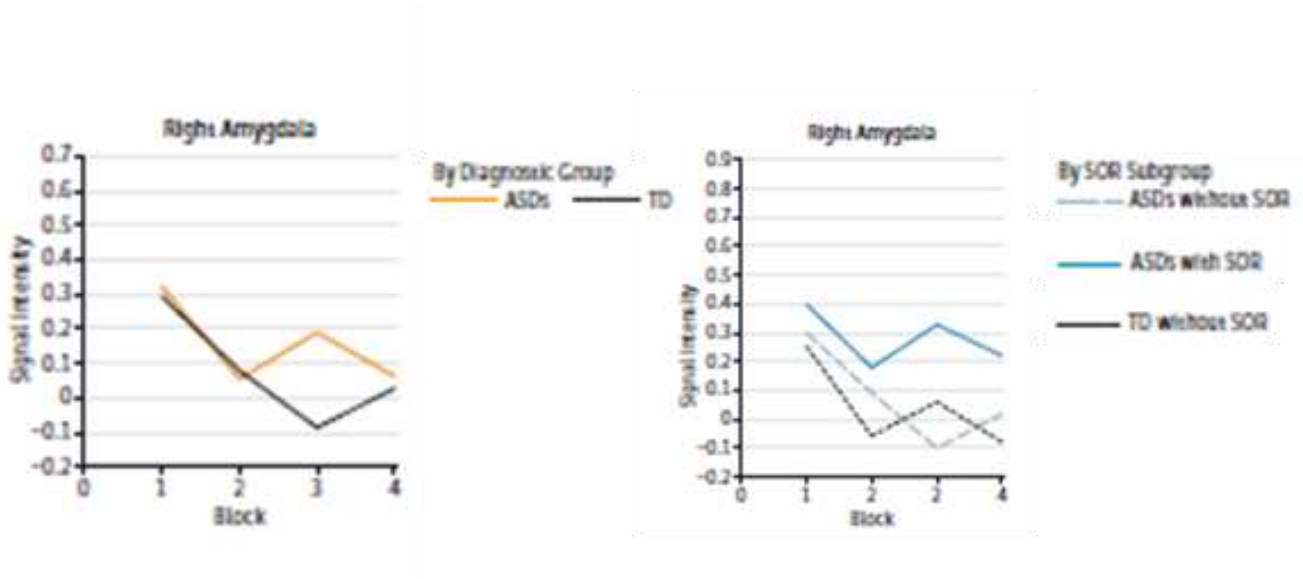

Figure 5: Decreased amygdala habituation is observed in the ASD group compared to TD controls is observed when presented with aversive stimuli (left). The ASD without SOR shows a stronger decrease in habituation compared to the ASD group with SOR and the TD group (Figure adapted from Green et al. 2015).

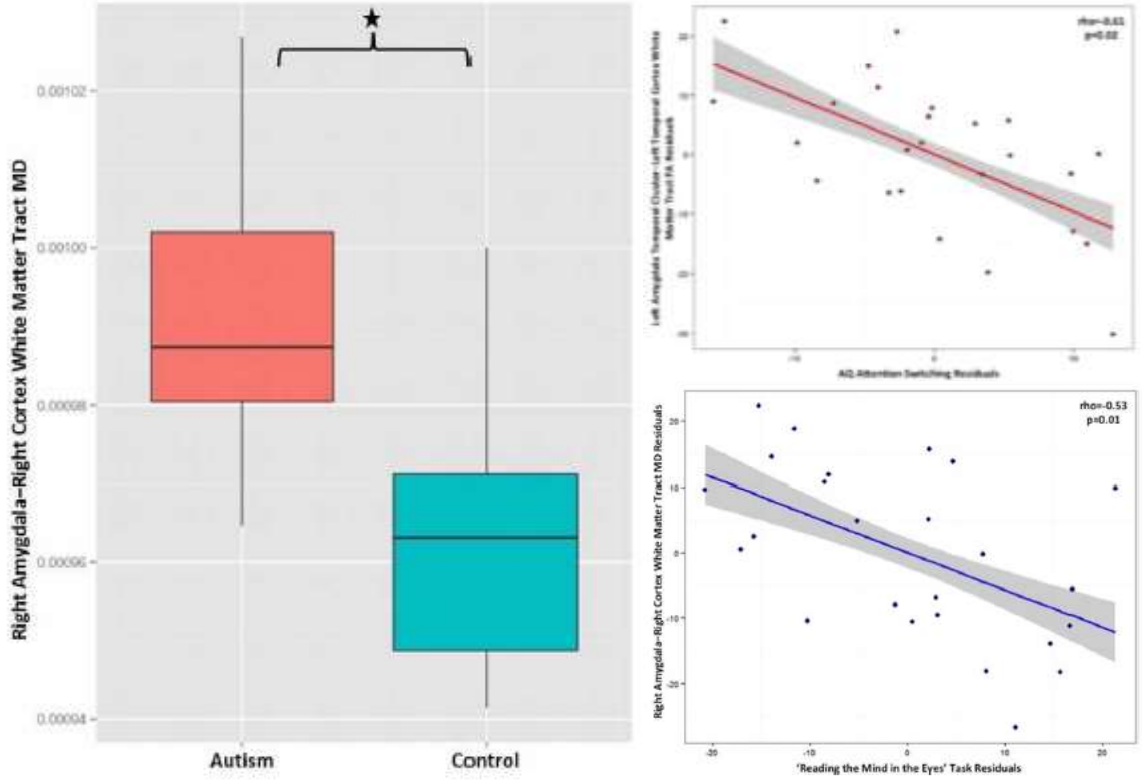

Figure 8: *(A) Mean diffusivity (MD) of white matter tracts connecting the right amygdala to the right cortex is significantly increased in ASD group (left) compared to controls (right). (B) Severity of symptoms in ASD adults (mean age ~24 years) negatively correlated to white matter tract fractional anisotropy (FA) residuals (top) and MD residuals (bottom) (Figure adapted from Gibbard et al. 2018).*

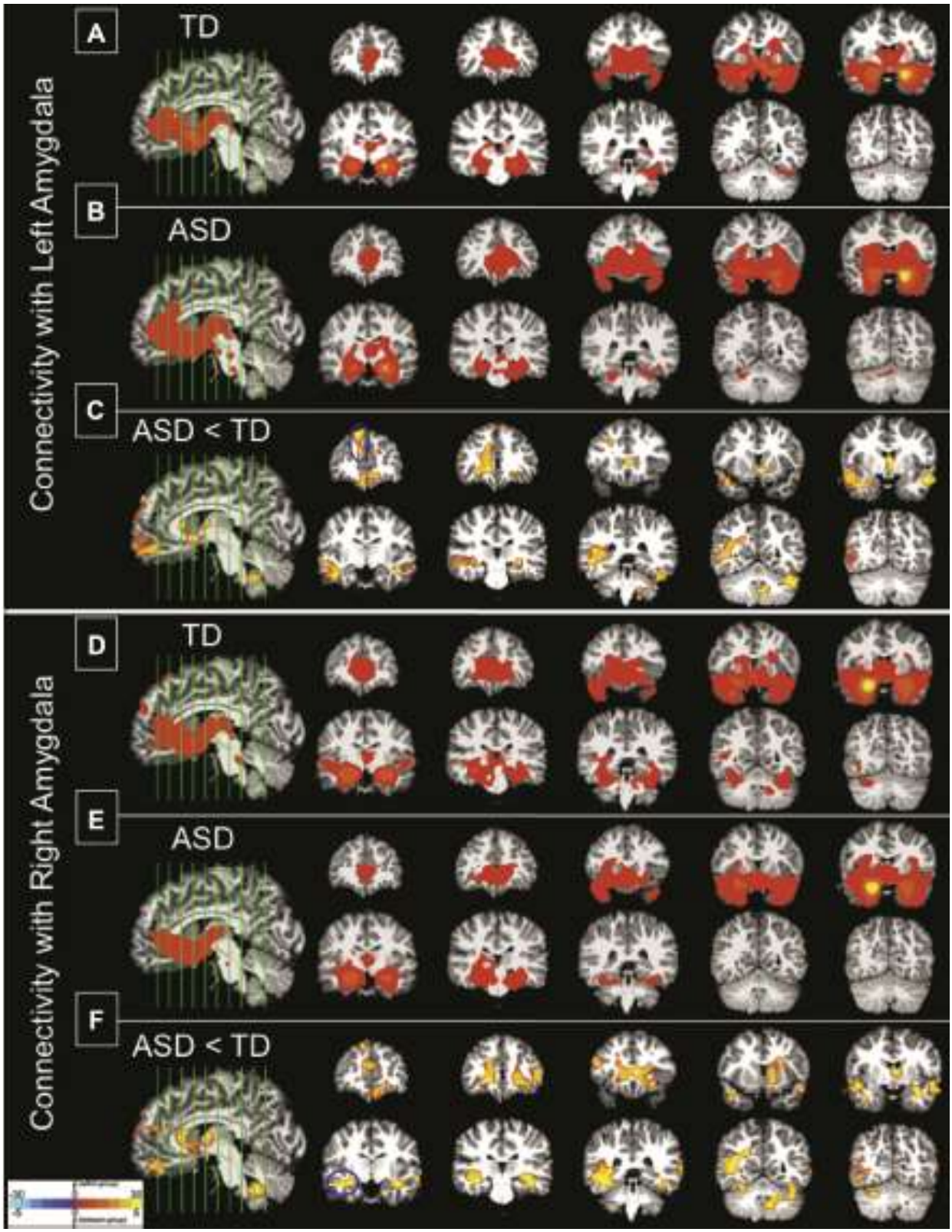

Figure 9: *MRI scans show ASD children (mean age 3.5 years) have weaker connectivity between the amygdala and brain regions involved in social and repetitive behaviors. (A and D) TD children (B and E) children with ASD (C and F) between-group differences. All significance $p < 0.05$ (Shen et al. 2016).*

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