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A Structural Examination of the Connection Between the Amygdala and Nucleus Accumbens in Adolescents with Clinical Anxiety

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Abstract

The prominence of anxiety disorders in today's general population is a major public health concern. Advancing research of the underlying pathophysiology of anxiety disorders can lead to the discovery of effective treatment interventions to treat the mental and physical symptoms of anxiety, and thus improve quality of life. This study aimed to examine two brain areas in the limbic and reward systems, the amygdala and Nucleus Accumbens (NAcc), and the structural white matter connection between them. This neural circuit assigns affective valence to environmental stimuli and motivates behavior to avoid potential harm. This study utilized diffusion Magnetic Resonance Imaging (dMRI) from the longitudinal multi-site Adolescent Brain Cognitive Development (ABCD) study to investigate the structure of these brain regions and the coherence of the white matter tract that directly connects them. Results indicated that in adolescents with clinical anxiety, significantly reduced Fractional Anisotropy (FA; an index of decreased neuron density, neuronal disorganization, and demyelination) and significantly reduced volume are observed in the BasoLateral nucleus of the Amygdala (BLA) in both the left and right hemispheres when compared to controls. This diffusion metric of FA was also found to be significantly correlated with volume in the BLA of both hemispheres. Additionally, in the BLA-NAcc tract of the left hemisphere, FA was significantly reduced in the first 25 nodes nearest the BLA for anxious participants compared to controls. Reduced FA within the BLA was also found to be significantly correlated with increased anxiety symptom severity. This study investigated structural differences between anxious and non-anxious adolescents during a period of maturation in which individuals are at an elevated risk of developing an anxiety disorder. The ABCD study is ongoing and provides the opportunity for future research to build from this presented study and investigate how anxiety may induce cognitive changes over time.

Introduction

Background

Anxiety disorders, including Generalized Anxiety Disorder (GAD), Social Anxiety Disorder (SAD), Panic Disorder (PD), Specific Phobias (SP), Obsessive-Compulsive Disorder (OCD), and PostTraumatic Stress Disorder (PTSD), are some of the most prominent psychiatric disorders afflicting society, affecting individuals of all age ranges and often throughout the life course unless treatment/intervention is received. The National Alliance on Mental Illness estimates that approximately 19% of adults in the United States and 7% of children and adolescents in the United States suffer from some form of an anxiety disorder (2017). Anxiety disorders are of particular concern because individuals often receive comorbid diagnoses of the aforementioned anxiety disorders (Burkhouse et al., 2020). Additionally, children and adolescents are in the "highest risk period for developing an anxiety disorder," which is an important consideration when choosing samples for investigation into anxiety pathophysiology (Burkhouse et al., 2020).

Generalized Anxiety Disorder (GAD) is typically described as fear, distress, worry, and avoidance that is "excessive" or greater than what a situation warrants (Shin & Liberzon, 2010). As for the more specific anxiety disorders, Social Anxiety Disorder (SAD) is described as an extensive fear of social situations, performances, and embarrassment, often leading to avoidance of social settings (Shin & Liberzon, 2010). Panic Disorder (PD), marked by spontaneous panic episodes with severe somatic symptoms, also leads to avoidance of situations the individual feels may trigger an attack (Rauch et al., 2003). Phobias are directed at an "innocuous stimuli or situation" that causes amplified fear and anxiety in an individual and includes avoidance of the

phobia's focus (Rauch et al., 2003). Obsessive-Compulsive Disorder (OCD) is marked by obsessive, intrusive thoughts in which the individual completes compulsions (ritualistic tasks) in an attempt to ease the thoughts (Shin & Liberzon, 2010). PostTraumatic Stress Disorder (PTSD) is characterized by intense hyperarousal and recollections of a specific event that was traumatizing to the individual; individuals with PTSD will often exhibit avoidance of triggers as not to be reminded of their trauma (Shin & Liberzon 2010).

Past Research

As anxiety disorders are ubiquitous, several neuroimaging studies have been conducted utilizing technology including functional (fMRI) and diffusion Magnetic Resonance Imaging (or Diffusion Tensor Imaging, DTI), as well as Positron Emission Tomography (PET), in an attempt to understand the specific brain regions and circuitry involved in and impacted by anxiety disorders. The amygdala's implication in anxiety disorders is noted early on in past literature due to its involvement in processing fear stimuli. The amygdala processes fear stimuli by assigning sensory input with an "emotional value," accomplished by the "formation of associations between neutral predictive stimuli and outcomes of positive or negative valence" (Calhoon & Tye, 2015). Projections from the BLA will then activate either "fear or reward pathways" depending on the "valence of these cues" (Calhoon & Tye, 2015). Early fMRI studies, as described in the review by Rauch et al., exhibited the involvement of the amygdala in the processing of fear and anxiety-provoking stimuli; participants were presented with fearful human faces or "emotionally valenced" images, and increased amygdala activation (far more significant than amygdala activation after the presentation of a neutral/happy face or image) was observed (2003). One PET study, described by Rauch et al., also measured amygdala activation by utilizing classical conditioning; participants learned to associate a shock (Unconditioned

Stimulus; US) with a video of a snake (Conditioned Stimulus; CS) and regional Cerebral Blood Flow (rCBF) changes in the amygdala were monitored upon stimuli presentation (2003). The study revealed that increased rCBF in the amygdala was correlated with "electrodermal activity changes," indicative of an anxious somatic state (Rauch et al., 2003). A PTSD-focused study discovered amygdala hyperresponsivity as central to PTSD pathophysiology; participants with PTSD (versus those with trauma exposure without clinical PTSD) experienced significantly greater amygdala activation, which was revealed through fMRI imaging, after being provoked by a reminder of their trauma (Rauch et al., 2003). In a PET study of individuals with SAD, participants were involved in a public speaking experiment and results indicated increased rCBF in the amygdala for individuals diagnosed with a social anxiety disorder (Rauch et al., 2003). Anxiety studies have also looked into volumetric and morphometric differences of Regions-Of-Interest (ROI); for example, "one large mMRI study found reduced orbitofrontal cortical and amygdala volumes in OCD vs. healthy control subjects" (Rauch et al., 2003). The review and study by Burkhouse et al. discusses that individuals with GAD, PD, PTSD, and SAD have been shown to possess a "decreased amygdala volume across youth and adult samples," revealed through past morphometric studies, and suggest that this volume reduction is likely due to "glutamatergic excitotoxicity" from the amygdala hyperactivity that is characteristic of anxiety disorders (2020). For their adult study, it was also discovered through statistical analyses that "greater baseline anxiety severity was associated with reduced left amygdala volume" (Burkhouse et al., 2020). Additional adult studies described by Burkhouse et al. have shown that anxiety symptom severity "is associated with larger NAcc volume across adults;" the Burkhouse et al. study replicated this finding and discovered that this morphometric abnormality was

indicative of a more positive response to anxiety treatment (cognitive behavioral therapy/selective serotonin reuptake inhibitor administration) post-study (2020).

The Nucleus Accumbens (NAcc), known for its involvement in motivation and reward processing, is slightly more inconspicuous in anxiety literature compared to other ROIs like the amygdala; yet, its functions are directly implicated in the avoidance behaviors characteristic of anxiety disorders. More recent findings, such as the volumetric abnormalities noted by Burkhouse et al., have highlighted NAcc involvement in the "motivation for avoidance behaviors" to anxiety-provoking stimuli (2020). The NAcc is involved in responding to aversive stimuli and then motivating "passive and active avoidance behavior," which directly corresponds to the typical behavior of persistent avoidance seen with anxiety disorders (Burkhouse et al., 2020). Animal literature has demonstrated NAcc involvement in avoidance through NAcc lesion studies; one study involving an "odor-guided discrimination task" found that the NAcc "integrates the motivational value of both appetitive and aversive cues" to direct a behavioral response as the lesioned rats failed to show expected changes in "response latency during discrimination learning" to the odor indicating the release of a foul-tasting solution (Schoenbaum & Setlow, 2003). Functional imaging in a study conducted by Levita et al. showed that during active avoidance of threat (emitting response by pressing a button), the NAcc exhibited increased activation, and during passive avoidance (omitting response by withholding from pressing the button), increased deactivation, indicative of NAcc involvement in harm avoidance (2012). Additionally, the participants' anxiety level at the time of the study was shown to impact the amount of activation/deactivation seen in the NAcc, with high anxiety correlating to both greater activation and deactivation for active and passive avoidance, respectively (Levita et al., 2012). This reveals the importance of considering the NAcc in anxiety pathophysiology and shows that

the NAcc has involvement in the constancy of avoidance behaviors in individuals with anxiety disorders (Levita et al., 2012).

The aforementioned studies focused on anxiety and the amygdala and NAcc as individual ROIs, but a few studies have also looked into the circuitry, or white matter connectivity, that is believed to play a role in anxiety disorders using diffusion MRI (dMRI). Diffusion MRI is utilized to predict the orientations of white matter tracts in the brain as water diffusion is "less hindered parallel than perpendicular to axons" (Jbabdi et al., 2015). dMRI measures "the diffusion of water molecules in a voxel in the brain from several directions" and uses an algorithm to generate a "diffusion tensor," which provides information about metrics like "fractional anisotropy" in that area (Ayling et al., 2012). Fractional Anisotropy (FA) is the measure most commonly used in white matter studies to compare the "integrity" of the white matter connections (Jbabdi et al., 2015). FA is a measure of the "variance of the diffusion coefficient along all directions in three dimensions under the assumption of anisotropic Gaussian diffusion," and can reveal important characteristics of white matter connections such as myelination/demyelination and density of tracts (Jbabdi et al., 2015). Thus, dMRI relies on the directionally dependent diffusion of water molecules in the brain and discerns differences in "neuronal organization" or tract coherence between subjects by allowing for the comparison of FA values (Ayling et al., 2012). This has been shown in animal models where mice lacking myelinated axons exhibit lower values of fractional anisotropy (Tisdall et al., 2022). Research on patient populations with anxiety has utilized a form of dMRI called Diffusion Tensor Imaging (DTI) and has focused primarily on major white matter tracts at the level of the entire brain. In the review and adolescent whole-brain white matter study by Liao et al., DTI results showed "reduced FA in the uncinate fasciculus" (UF), which connects limbic regions, including the

amygdala, to the PreFrontal Cortex (PFC) and Cingulate Cortex (CC), for adolescents with GAD (2014). The researchers concluded that this abnormal tract coherence of the UF could be an explanation for the elevated amygdala activation seen in individuals with anxiety (Liao et al., 2014). Reduced FA in the UF was also observed in additional studies involving adults diagnosed with GAD (Liao et al., 2014). The DTI study by Liao et al. also found lower FA values in the Inferior Longitudinal Fasciculus (ILF) of adolescents with generalized anxiety, which connects structures including the amygdala and hippocampus, and this was also seen in an external study in older adults with GAD (2014). The researchers involved in this adolescent study conducted an additional white matter study in adults that revealed "higher FA in the right amygdala white matter and lower FA in the caudal anterior cingulate cortex white matter" in individuals with anxiety (Liao et al., 2014). The Anterior Cingulate Cortex (ACC) is connected to limbic system regions, including the amygdala and NAcc (Sturm et al., 2016). The finding of higher FA in the right amygdala is interesting as other white matter studies indicate that they did not find regions of increased FA in participants with anxiety; however, this study was disadvantaged by an especially small sample size of only 16 participants. In a DTI study conducted by Wang et al., low FA values were seen in the following major white matter "clusters" in psychiatric anxiety patients: "bilateral uncinate fasciculus, body of corpus callosum, left middle cingulum (cingulate gyrus), bilateral anterior thalamic radiation and corona radiate, right anterior limb of internal capsule, bilateral inferior frontal-occipital fasciculus, bilateral superior and inferior longitudinal fasciculus" (2016). Wang et al. also elucidate that the low FA values of the UF and cingulum were associated with greater anxiety symptom severity in the patients studied (Wang et al., 2016). Another DTI study looking specifically at the amygdala-to-PFC connection also stated that the integrity of this connection is related to reported anxiety levels, explicitly, "higher

pathway strength predicted lower anxiety," referring to reported trait anxiety (Kim & Whalen, 2009). The lower FA values observed in these studies can be explained by numerous factors, including decreased density/myelination of axons, "abnormal axonal membranes," and "reorganization of axons at a macroscopic level," characteristically, disorganization (Lu et al., 2018). Opposingly, FA values that are higher than normal "are thought to reflect a higher degree of neuronal organization" (Ayling et al., 2012).

Whereas previous research measured the major long-range white matter tracts of the whole brain, less research has targeted white matter tracts between two specific brain loci. Additionally, previous white matter studies of individuals with anxiety disorders have not focused in on the reward system and its white matter connections, such as the amygdala to NAcc tract, which has been more recently known to play a role in anxiety disorders in regards to avoidance behavior. A few studies have taken a functional look at this amyg-NAcc connection; the study by Levita et al. showed an "increase in functional connectivity between the amygdala and NAcc" while active avoidance was occurring in individuals with anxiety (2012). Since the NAcc is an output of the amygdala, the researchers believe that increased amygdala output, occurring from amygdala hyperresponsivity typical of an anxiety disorder, could explain this observation (Levita et al., 2012). An animal study involving mice conducted by Beyeler et al. also took a functional look at the amygdala-NAcc connection by observing the activity of photo-identified neurons using classical conditioning techniques (2016). The mice were trained to discriminate between two sounds and associate the sounds with either a positive or negative outcome; the researchers found that 80% of neurons projecting from the basolateral amygdala to the NAcc encoded positive valence (i.e., reward) (Beyeler et al., 2016). The involvement of the NAcc in reward processing is applicable to studies of anxiety disorders, but SAD or social

phobias are particularly of interest due to the theory of "dysregulated reward" acting as a cognitive component leading to "social deficits" (Rauch et al., 2003). SAD is reported to be "the third most common mental health disorder after depression and substance abuse," and children and adolescents are of special concern as 90% of individuals with social anxiety experience this disorder before the age of 23 (Leigh & Clark, 2018).

The previously mentioned ROIs that show volumetric changes in individuals with an anxiety disorder are "areas where glutamate neurons predominate," so it is hypothesized that increased glutamate release is "crucial for structural/functional changes" (Musazzi et al., 2013). The review by Musazzi et al. elucidates this mechanism of "maladaptive changes" by explaining that when glutamate transmission is hyperactive, this leads to "building up excessive synaptic or extrasynaptic levels of glutamate," which can result in aberrant neuronal remodeling (2013). Selective Serotonin Reuptake Inhibitors (SSRIs), however, are antidepressants hypothesized to "exert a protective action when the synapse is overactivated by stress-related mechanisms" (Musazzi et al., 2013). This theory comes from animal studies, for instance, it has been observed that the SSRI fluoxetine "reduced depolarization-evoked release of glutamate" in hippocampal "superfused synaptosomes" (Bonanno et al., 2005). The review by Musazzi et al. also explains that the association "between dendritic remodeling and volumetric changes is at present inferential," which provides an interesting area of future research in anxiety pathophysiology (2013).

This research project will take a more microscopic look at two ROIs in anxiety pathophysiology, the amygdala and nucleus accumbens, and their white matter connection at the structural, rather than functional, level using obtained baseline diffusion MRI data from the Adolescent Brain Cognitive Development (ABCD) study. This research will utilize the diffusion

metric of fractional anisotropy to investigate neuronal organization within and between these ROIs for the following groups of ABCD participants: those with a clinical level of anxiety who are not on SSRIs, those with a clinical level of anxiety who are on SSRIs, and those who do not have a clinical level of anxiety and who are not on SSRIs (controls). Due to the findings of the presented past research studies, and the knowledge that within the reward pathway the amygdala sends glutamatergic inputs to the NAcc, I hypothesize that the ABCD participants studied with clinical anxiety will exhibit abnormalities in white matter tract coherence between the amygdala and NAcc, indicated by decreased FA, and that this neuronal disorganization will also be seen within the amygdala and NAcc ROIs. I also hypothesize that SSRI treatment will provide FA values similar to control participants, indicative of the medication's protective effects on glutamatergic excitotoxicity. Building from past literature, I also presume that reduced amygdala volume will be found in ABCD participants with a clinical level of anxiety, and the volumetric and diffusion metric differences for this anxious group should also be associated with the individual's level of anxiety symptom severity.

Method

Participants

The Adolescent Brain Cognitive Development study is a longitudinal study funded by the NIH that aims to discover how the brain changes and develops over the period of adolescence and how neural development is influenced by external factors such as substance use. For the baseline year, the ABCD study recruited 11,876 participants between 9 and 10 years of age. These participants were assessed for possible mood, behavioral, and psychiatric disorders using the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) coupled with the Child

Behavior Checklist (CBCL), which is "a standardized form that assesses parent-reported behaviors, problems, and competencies in youth ages 4–18" (Kendall et al., 2007). The participants were assigned a *t*-score correlated with their symptom severity for various disorders, including anxiety, with a *t*-score of 60 and over indicating a clinical-level disorder. Hence, baseline participants can be divided into anxiety-disordered ("anxious") and non-anxiety-disordered ("controls") categories. Further, the ABCD study obtained data concerning all medications its participants were taking at baseline. The anxiety-disordered group can thus be divided into a medicated subgroup ("SSRI"), indicative of adolescents with clinical anxiety who were taking any of the five FDA-approved SSRIs at baseline (Citalopram, Escitalopram, Fluoxetine, Paroxetine, Sertraline), and a NOn-Medicated subgroup ("NOM"). For this research study, the baseline sample size was narrowed down to participants who underwent dMRI scanning using Siemens scanners, as these consistently provided the best diffusion MRI results. Further, subjects were eliminated on the basis of excessive head movement while undergoing scans to eliminate noisy data; accordingly, 4,055 baseline participants with sufficient dMRI data were obtained for further analyses. Within these 4,055 participants, 581 fell into the "anxious" category, 31 into the "SSRI" category, 550 into the "NOM" category, and 3,443 into the "controls" category.

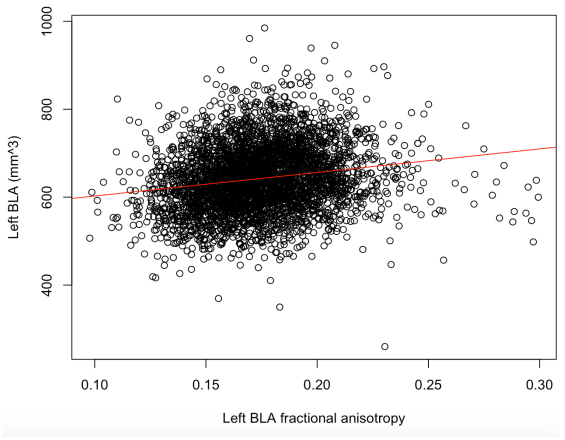
Procedures

For image preprocessing, the MATLAB mrDiffusion package was utilized. The ABCD baseline 3D-T1 weighted structural MRI data underwent AC-PC alignment, meaning the Anterior and Posterior Commissures (major white fiber tracts) and midsagittal plane were marked as coordinates in the brain to adjust the T1 images for head tilt, and subsequently transform the T1 images into "AC-PC aligned space" (Leong et al., 2016). The baseline ABCD

diffusion MRI data was then transformed into this AC-PC aligned space. The AC-PC aligned data was processed with FreeSurfer software in order to define the amygdala and NAcc brain regions as Volumes-Of-Interest (VOIs) using a voxel-based "binary mask" that excluded anatomically inaccurate voxels out of the masked regions. For this study, special emphasis is placed on the basolateral amygdala as a VOI because the amygdala-to-NAcc connection is based from the basolateral nucleus of the amygdala (Kamali et al., 2015). For the amygdala to NAcc connection, MRtrix software was used to perform "constrained spherical deconvolution based probabilistic tracking" (Leong et al., 2016). The spherical deconvolution tracking method is used to "estimate the distribution of fibre orientations present within each imaging voxel," even in voxels that may have "contributions from differently oriented fibre bundles" (Tournier et al., 2007). Fiber generation was then accomplished by "seeding a voxel" in either the amygdala or NAcc VOI and "tracking until the fiber reached the ending VOI" around a million times (Leong et al., 2016). To obtain a core tract and eliminate outliers, another binary mask, representative of the anatomically appropriate white matter route from the amygdala to NAcc, was overlaid to cancel the inaccurately generated fibers that left the mask. The binary mask for the tract was generated from automated outlier trimming followed by manual adjustment trimming in MATLAB from an independent study of 216 participants. The generated amygdala to NAcc fiber tract was then divided into 100 even "nodes" to calculate the mean FA per node by taking an "average of each fiber's FA in that node, weighted by the spatial distance of that fiber from the node's core fiber" (Leong et al., 2016). This division allows investigation into differences in FA along the entire trajectory of the white matter tract, which is important when also investigating neuronal organization within and near associated VOIs.

Results

To test for significant differences in FA in the right and left BLA VOIs, various two-sample *t*-tests were completed for the following groups: anxious vs controls, SSRI vs controls, NOM vs controls, and NOM vs SSRI. The anxious vs controls *t*-tests revealed FA is significantly reduced in the right ($t(862) = -1.65, p = 0.05$) and left ($t(897) = -2.70, p = 0.004$) BLA VOIs for participants with clinical anxiety compared to non-anxious participants. The *t*-tests for SSRI vs controls were insignificant for FA differences in the right ($t(31) = 1.06, p = 0.30$) and left ($t(31) = -0.96, p = 0.30$) BLA VOIs between the two groups. The NOM vs controls *t*-tests showed FA is significantly reduced in both the right ($t(804) = -1.96, p = 0.03$) and left ($t(825) = -2.62, p = 0.005$) BLA regions for participants with a clinical level of anxiety who are not on an SSRI. The NOM vs SSRI *t*-tests showed marginal significance for reduced FA in the right BLA for non-medicated anxious participants compared to medicated participants ($t(33) = -1.50, p = 0.07$), but not for the left BLA region ($t(37) = -0.09, p = 0.5$). To further elaborate on the significantly reduced FA observed in the right and left BLA for the anxious and NOM participants, the following linear regressions were run (*Figure 1*). Results showed that the FA diffusion metric is significantly correlated with BLA volume in mm^3 for both hemispheres: right ($R^2 = 0.03, F(1, 3785) = 97.69, p < 0.001$) and left ($R^2 = 0.03, F(1, 3786) = 122.10, p < 0.001$).



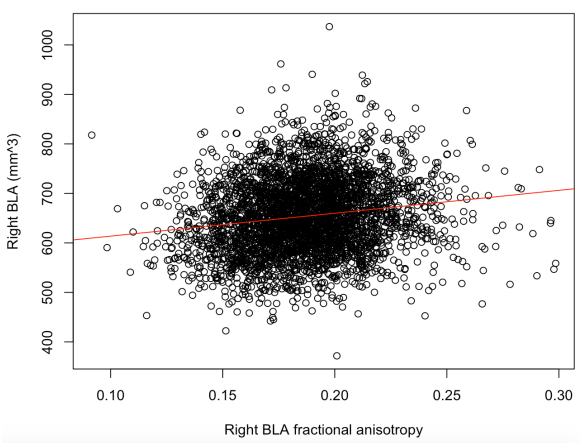


Figure 1. *Structural coherence within the BLA is correlated with greater volume (Fractional Anistropy; FA; Left:* R^2 *= 0.03, F(1, 3786) = 122.10, p < 0.001; Right:* R^2 *= 0.03, F(1, 3785) = 97.69, p < 0.001).*

Additional *t*-tests were conducted for the four comparisons to investigate volumetric differences in mm^3 for the right and left BLA. The anxious vs controls *t*-tests results revealed that BLA volume is significantly reduced in anxiety-disordered participants compared to non-disordered participants in both the right (*t(792) = -2.30, p = 0.01*) and left (*t(784) = -1.82, p = 0.03*) hemispheres. The SSRI vs controls *t*-tests indicated reduced BLA volume for anxiety-disordered participants on an SSRI in the right hemisphere (*t(31) = -2.15, p = 0.04*), but not in the left (t(30) = -1.35, p = 0.2). The NOM vs controls *t*-tests showed that volume is reduced in the BLA in anxiety-disordered participants who do not take SSRIs in both hemispheres: right, significantly, (*t(737) = -1.95, p = 0.03*) and left, marginally, (*t(732) = -1.59, p = 0.06*). The NOM vs SSRI *t*-tests did not indicate significantly reduced BLA volume for non-medicated anxious participants when compared to medicated: right (*t(35) = 1.52, p = 0.9*),

left (*t(33) = 0.91, p = 0.8*). The previously mentioned BLA statistical tests for FA and volumetric differences per hemisphere are summarized in *Table 1*.

	Right BLA FA	Right BLA Volume	Left BLA FA	Left BLA Volume
Anxious vs controls	t(862) = -1.65, p = 0.05*	t(792) = -2.30, p = 0.01*	t(897) = -2.70, p = 0.004*	t(784) = -1.82, p = 0.03*
SSRI vs controls	t(31) = 1.06, p = 0.3	t(31) = -2.15, p = 0.04*	t(31) = -0.96, p = 0.3	t(30) = -1.35, p = 0.2
NOM vs controls	t(804) = -1.96, p = 0.03*	t(737) = -1.95, p = 0.03*	t(825) = -2.62, p = 0.005*	t(732) = -1.59, p = 0.06*
NOM vs SSRI	t(33) = -1.50, p = 0.07*	t(35) = 1.53, p = 0.9	t(37) = -0.09, p = 0.5	t(33) = 0.91, p = 0.8

Table 1. Summary of BLA-focused statistical tests.

Two-sample *t*-tests were repeated for the four comparisons to test for a significant reduction of FA in the right and left NAcc VOIs. The anxious vs controls *t*-tests were insignificant for FA differences in the right (*t(774) = 1.45, p = 0.2*) and left (*t(792) = 1.59, p = 0.1*) NAcc for anxiety-disordered participants. The SSRI vs controls *t*-tests were also insignificant in the right (*t(29) = 1.49, p = 0.2*) and left (*t(29) = 1.30, p = 0.2*) NAcc for FA differences in medicated anxiety-disordered participants compared to non-disordered. FA was also not found to be significantly altered in the right (*t(727) = 1.14, p = 0.3*) or left (*t(742)= 1.34, p = 0.2*) NAcc for anxiety-disordered participants not on SSRIs compared to non-disordered participants. No significant NAcc FA differences were found in either hemisphere when comparing the NOM vs SSRI groups: right (*t(32) = -1.22, p = 0.2*), left (*t(32) = 0.97, p = 0.3*).

The between-group tests for volumetric differences were repeated for the left/right NAcc (the tests were not run with directionality as NAcc volume changes in anxiety pathophysiology are more inconspicuous in the literature). The anxious vs controls *t*-tests showed marginal significance for an increased volume in anxious participants for the right (*t(808) = 1.82, p = 0.07*) NAcc, but not for the left (*t(802) = 1.47, p = 0.1*). SSRI vs controls *t*-tests did not show significant volumetric differences in the right (*t(30) = 1.00, p = 0.3*) or left (*t(30) = 0.66, p = 0.5*) NAcc, and neither did the NOM vs controls *t*-tests: right (*t(754) = 1.67, p = 0.1*), left (*t(748) = 1.38, p = 0.2*). The NOM vs SSRI *t*-tests also did not reveal volumetric differences in the right (t(33) = -0.55, p = 0.6) or left (t(33) = -0.27, p = 0.8) NAcc for non-medicated anxious participants versus medicated. The previously mentioned NAcc statistical tests for FA and volumetric differences per hemisphere are summarized in *Table 2*.

	Left NAcc FA	Left NAcc Volume	Right NAcc FA	Right NAcc Volume
Anxious vs controls	t(792) = 1.59, p = 0.1	t(802) = 1.47, p = 0.1	t(774) = 1.45, p = 0.1	t(808) = 1.82, p = 0.07*
SSRI vs controls	t(29) = 1.30, p = 0.2	t(30) = 0.66, p = 0.5	t(29) = 1.49, p = 0.1	t(30) = 1.00, p = 0.3
NOM vs controls	t(742) = 1.34, p = 0.2	t(748) = 1.38, p = 0.2	t(727) = 1.14, p = 0.3	t(754) = 1.67, p = 0.1
NOM vs SSRI	t(32) = 0.97, p = 0.3	t(33) = -0.27, p = 0.8	t(32) = -1.22, p = 0.2	t(33) = -0.55, p = 0.6

Table 2. Summary of NAcc-focused statistical tests.

Two linear regressions were performed to discover if anxiety symptom severity, indicated by a higher CBCL/DSM-5 derived *t*-score, is correlated with FA values in the right and left BLA. For the right BLA, results revealed that higher FA is correlated with a reduced *t*-score, or less

anxious symptoms, as seen in *Figure 2* (R^2 = *0.001, F(1, 3859) = 4.66, p = 0.03*). For the left BLA, results showed higher FA is also correlated with reduced *t*-scores or lower anxiety symptom severity, as seen in *Figure 2* (R^2 = *0.001, F(1, 3875) = 4.74, p = 0.03*).

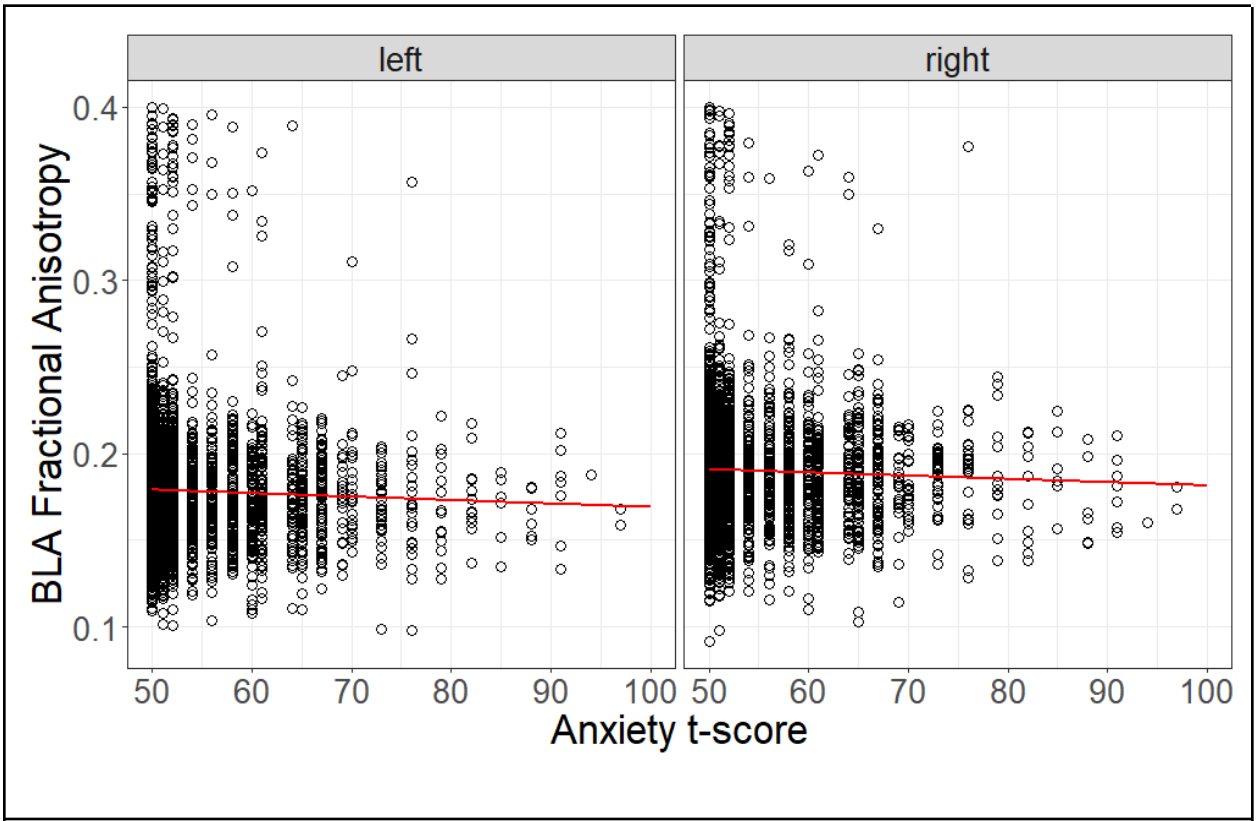


Figure 2. *Structural coherence within the BLA is correlated with reduced anxious symptom severity (Fractional Anistropy; FA; Left:* R^2 = *0.001, F(1, 3875) = 4.74, p = 0.03; Right:* R^2 = *0.001, F(1, 3859) = 4.66, p = 0.03)*

Linear regressions were also performed to investigate a possible correlation between anxiety symptom severity (*t*-score) and BLA volume. In the right hemisphere (*Figure 3*), BLA volume is moderately correlated with anxiety symptom severity (R^2*= 0.001, F(1,3859) = 3.51, p*

= 0.06), but this correlation is insignificant in the left hemisphere as seen in *Figure 3* (R^2 = *0.001, F(1, 3875) = 2.78, p = 0.1*).

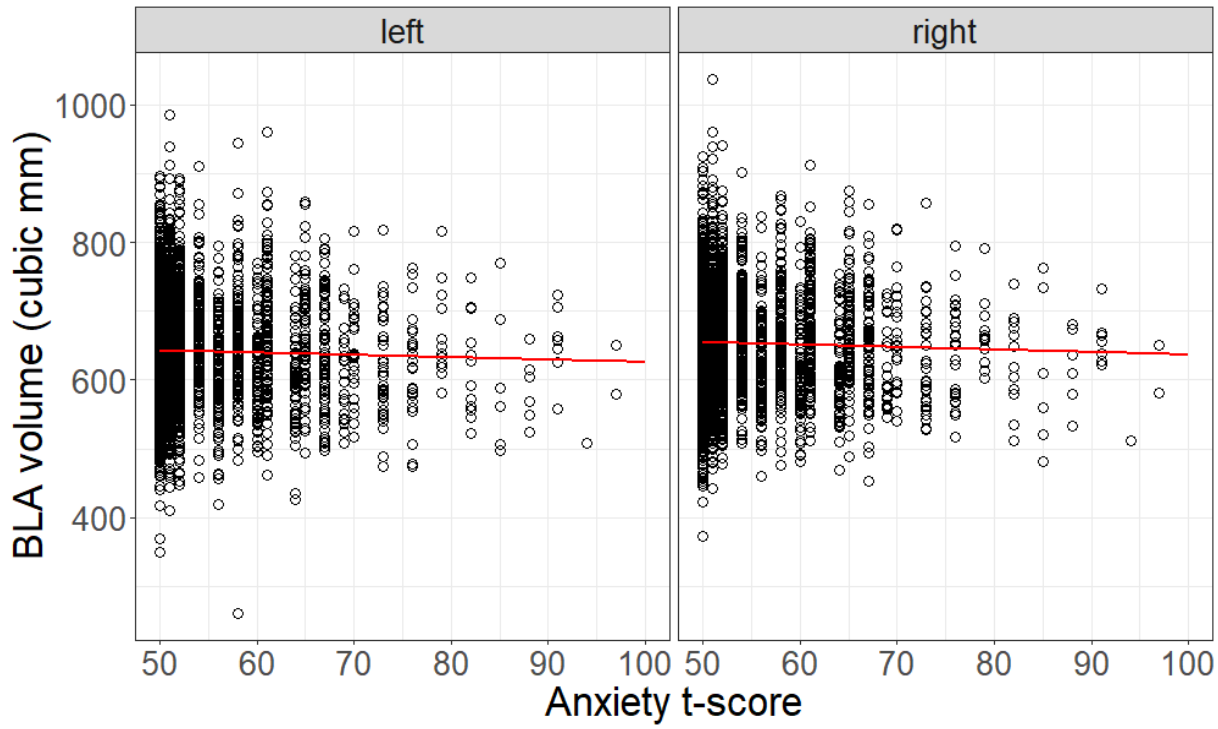


Figure 3. *Greater BLA volume in the right hemisphere is moderately correlated with reduced anxious symptom severity (Left: R^2 = 0.001, F(1, 3875) = 2.78, p = 0.1; Right: R^2 = 0.001, F(1,3859) = 3.51, p = 0.06)*

The following plot (*Figure 4*) shows the differences in FA across the entire white matter tract from the BLA to the NAcc for both hemispheres for controls vs anxious participants. Visually, it appeared possible that a significant FA difference could exist between the grouped participants for the first 25 nodes (nearest the BLA) for the left hemisphere. To investigate, a

two-sided *t*-test was used and revealed that anxiety-disordered participants had significantly reduced FA values within the range of the first 25 nodes when compared to the control (non-anxiety) group for the left hemisphere (*t(786) = 2.74, p = 0.006*).

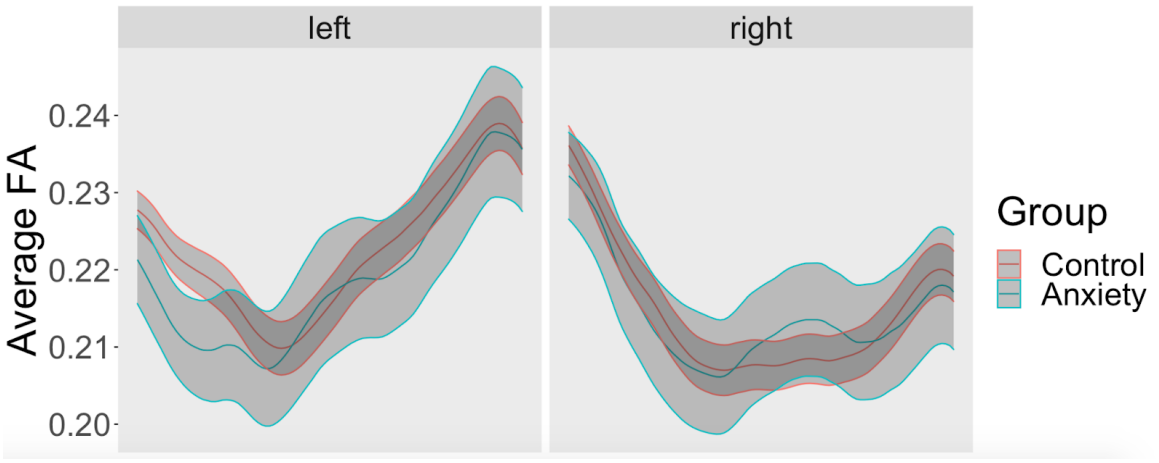


Figure 4. *Anxious participants show reduced structural integrity in the first 25 nodes of the amyg-NAcc white matter connection in the left hemisphere (Fractional Anistropy; FA; Left: t(786) = 2.74, p = 0.006).*

Discussion

The purpose of this study was to comprehensively characterize two important brain areas implicated in anxiety disorders, the amygdala and nucleus accumbens, as well as their white matter connection during a critical period of early adolescence. The consensus in past anxiety literature of reduced amygdala volume and reduced FA in the major white matter tracts that connect with limbic structures, and the theories of amygdala hyperactivity and associated glutamate excitotoxicity as a cause of aberrant neuronal remodeling, led me to hypothesize that reduced FA will be observed within the amygdala and NAcc ROIs and along their connecting

white matter tract when compared to adolescents without clinical anxiety. Animal literature investigating the protective actions of SSRIs also led me to presume that the medicated ABCD participants with clinical anxiety would show FA values and ROI volumes that more closely resembled controls than non-medicated anxious participants. Morphometric changes of the NAcc are underreported in anxiety literature, but as the NAcc is a major glutamatergic output of the amygdala, I presumed that similar FA and volumetric reductions would be seen in this ROI, as in the amygdala, in ABCD participants with clinical anxiety.

As predicted, significantly reduced FA and reduced volume in mm^3 were observed in the BLA in both hemispheres in ABCD participants with an anxiety disorder when compared to controls; these findings coincide with aforementioned past research such as the work by Rauch et al. (2003) and Burkhouse et al. (2020). When looking at the subcategories of the anxious group, the non-medicated anxious participants showed a decrease in BLA volume (right hemisphere was significant, left was marginal) and a significant decrease in FA in the BLA of both hemispheres when compared to controls. While the ABCD participants being treated with SSRIs showed a reduced right BLA volume, an insignificant difference in BLA FA values was observed for both hemispheres when compared to controls. This indicates that SSRI administration may exert a protective role structurally within the amygdala, as the SSRI group FA values more closely resembled controls. However, the FA differences were not drastic enough to give a significant difference in either hemisphere when the medicated anxious participants were compared to the non-medicated anxious participants by *t*-tests (only marginal significance in the right BLA was observed). Additionally, SSRI consumption did not appear to confer any volumetric differences. Although FA is a measure commonly used to decipher white matter tract integrity, looking at changes in FA within brain regions like the amygdala can provide

information about the organization of neurons within that region, as well as information about dendritic remodeling, like dendritic spine formation/reduction, for example. As explained by Musazzi et al., volumetric changes due to dendritic remodeling are "inferential," and it was this statement that prompted my investigation into the correlation between FA and volume of the BLA; the results indicated a highly significant ($p < 0.001$) correlation, revealing that lower FA is associated with a lower BLA volume, and vice versa (2013). Lower FA is associated with decreased neuronal density, so lower FA within the BLA may be attributed to "decreased dendritic spine density" (Kubota et al., 2013). Hence, this FA-to-volume correlation likely provides evidence that dendritic remodeling is an underlying mechanism of volumetric changes of specific ROIs, in this instance, the BLA. However, dendritic remodeling likely is not the only underlying mechanism of the observed volumetric changes in the amygdala in participants with clinical anxiety. The amygdala contains glutamatergic projections between its own nuclei (the BLA projects to the central nucleus, for example), so decreased FA within the amygdala ROI could be attributed to diminished integrity of these projecting tracts, and the conjecture of glutamatergic excitotoxicity within the amygdala provides a mechanism as to how this aberrant change may occur (Siegle et al., 2006). Thus, this finding provides important evidence of a link between a structural diffusion metric implicating more macroscopic volumetric changes in the brain, but further research would be beneficial to undoubtedly confirm the exact mechanisms at play that result in the major gray matter volumetric differences seen in the amygdala of both adolescents and adults with clinical anxiety.

Though no significant FA differences were found between groups for the NAcc ROI, there was a marginally significant increase in right NAcc volume observed in anxious ABCD participants compared to controls. This is seen in a few anxiety studies such as the Burkhouse et

al. study, which reported that in adults anxiety symptom severity is positively correlated with larger NAcc volumes (2020). Investigating volumetric changes in the NAcc during adolescence is an area for continued research, and special emphasis should be placed on the later years of adolescence to see if volumetric changes occur that replicate the prior mentioned adult findings. This could be done through further analysis of the ABCD participants as this longitudinal trial progresses and more annual data is released. This opportunity for continued study of NAcc involvement in anxiety pathophysiology throughout the adolescent period is particularly interesting because of the increased plasticity of the brain during this stage of human development. This study utilized baseline 9-10 year olds, and this age may be too early on in the life course to observe changes in the NAcc in anxious individuals that could possibly occur from amygdala hyperresponsivity and irregular tract integrity.

Investigation of FA differences along the white matter amygdala-to-NAcc tract were focused on the first 25, middle 50, and final 25 nodes. Within the first 25 nodes of the left hemisphere tract, a significant reduction in FA was observed for ABCD participants with a clinical level of anxiety. Though this FA reduction coincides with the findings within the amygdala, and these first 25 nodes are nearest to the BLA, some important limitations of generating the white matter tract should be discussed. Constrained spherical deconvolution based probabilistic tracking is an error-prone process; oftentimes more than one fiber tract will be automatically generated, or fibers that are anatomically impossible may be generated. Automated outlier trimming, masking, and manual fiber trimming are ways to fix or delete these erroneous fibers; but unfortunately, with a sample size as large as the ABCD study, manual fiber trimming is simply not feasible, so there is the possibility of some fiber generation errors going unnoticed. Despite this limitation, dMRI still provides crucial information about tract integrity and

myelination that is important to consider when investigating anxiety pathophysiology, as diminished axonal integrity/myelination is "suggested to lead to neuronal deficits such as alterations in synaptic function" (Kubota et al., 2013). Thus, reduced FA along the amyg-NAcc tract indicates the possibility for future dysregulation from the amygdala neuron projections that synapse within the nucleus accumbens. Future research that utilizes an older sample size could investigate this possible effect as the 9-10 year-olds of the baseline year of the ABCD study may not have experienced an anxiety disorder prolonged enough to generate structural changes along an entire white matter tract, assuming the white matter tract differences are the result of a cognitive process like neurotransmitter toxicity versus an inherent genetic predisposition.

The study by Burkhouse et al. that reported reduced amygdala volume in patients with anxiety also found that symptom severity was inversely correlated with left amygdala volume; however, the linear regressions I performed to investigate an association of BLA volume with symptom severity did not replicate this finding (2020). A white matter study by Wang et al. (2016) found a significant inverse relationship between UF and cingulum FA values and symptom severity, while another white matter study of the amyg-PFC connection by Kim & Whalen (2009) also reported a significant inverse relationship between tract FA and anxiety severity. The linear regressions I ran for both hemispheres to investigate the FA association with anxiety symptom severity also found that FA in the BLA is significantly, inversely correlated with anxious symptoms when restricting at an FA value of 0.4. As seen in *Figure 2,* participants within the 0.25-0.4 FA metric range appear to skew this correlation; yet, it seems unreasonable to restrict the FA metric further as this would exclude numerous participants rather than a few extraneous outliers. Moreover, FA reduction amongst white matter connections, especially those

that link limbic structures, is a common theme in anxiety literature, as well as associated increased symptom severity.

Another interesting area of future research is the protective effects of SSRIs at the structural level in individuals with an anxiety disorder; an investigation into this effect would be best represented longitudinally, and the ABCD study will provide this opportunity for analysis in the future. Future research could use the dMRI data released by the ABCD study to follow SSRI-taking participants throughout adolescence and compare these individuals to non-medicated participants that remain at a clinical level of anxiety throughout adolescence to investigate how this intervention mediates neuronal structural changes. Future research that compares anxious adolescents who take SSRIs to those that receive other interventions like cognitive behavioral therapy would also be extremely beneficial to identify the most effective treatment option that may reduce the prominence of anxiety in the adolescent population. Within the almost 12,000 participants of the baseline year, 14.7% of these young adolescents were rated as having a clinical level of anxiety. This is an alarming percentage, so effective treatment interventions need to be identified and implemented early in the life course in hopes to reduce the prominence of anxiety in future society. Though the ABCD study's large sample size brought about difficulty in image processing and tractography, the large sample size makes this study more applicable to the general public. Additionally, although the ABCD study did not partition the adolescents based on a specific anxiety disorder, and rather used a generalized rating/score, this also makes the study more applicable to society due to the prominence of comorbidity of either multiple anxiety disorders or anxiety and depression. Although this study only analyzed baseline data, the ABCD study will provide the opportunity for continued anxiety research due to

its longitudinal nature and provides the opportunity to further investigate cognitive changes that occur in adolescents with clinical anxiety over time.

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