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Journal

Biological Psychiatry, 86(12)

ISSN

0006-3223

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Publication Date

2019-12-01

DOI

10.1016/j.biopsych.2019.07.022

Peer reviewed

HHS Public Access

Author manuscript

Biol Psychiatry. Author manuscript; available in PMC 2020 December 15.

Published in final edited form as:

Biol Psychiatry. 2019 December 15; 86(12): 890–898. doi:10.1016/j.biopsych.2019.07.022.

The Relationship Between the Uncinate Fasciculus and Anxious Temperament is Evolutionarily Conserved and Sexually Dimorphic

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Abstract

Background—Anxious temperament (AT) is an early-life heritable trait, that predisposes individuals to develop anxiety and depressive disorders. Our previous work in pre-adolescent children suggests alterations in the uncinate fasciculus (UF), the white matter tract that connects prefrontal with limbic regions, in boys with anxiety disorders. Here, using a nonhuman primate model of AT, we test whether this sexually dimorphic finding is evolutionarily conserved, and examine the extent to which heritable and environmental influences contribute to UF microstructure.

Methods—Diffusion tensor images were collected in 581 young (43.9% female; 1.89+/−0.77 years) rhesus monkeys. Using tract-based analyses, the relationship between AT, UF microstructure as measured with fractional anisotropy (FA) and sex was assessed. Heritability of tract microstructure was determined using oligogenic linkage analysis of this large multi-generational pedigree.

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Disclosures

Dr. Alexander is part owner of Thervoyant. At the time of writing, Dr. Kalin had received honoraria from CME Outfitters, Elsevier, and the Pritzker Consortium; served on scientific advisory boards for Actify Neurotherapies, Neuronetics, and currently serves as an advisor to the Pritzker Neuroscience Consortium and consults to Corcept Therapeutics; served as co-editor of *Psychoneuroendocrinology,* and currently serves as Editor-in-Chief of *The American Journal of Psychiatry;* and has patents on promoter sequences for corticotropin-releasing factor CRF2alpha and a method of identifying agents that alter the activity of the promoter sequences (7,071,323; 7,531,356), promoter sequences for urocortin II and the use thereof (7,087,385), and promoter sequences for corticotropin-releasing factor binding protein and the use thereof (7,122,650). The other authors report no biomedical financial interests or potential conflicts of interest.

Results—We predicted and found, a negative relation between AT and UF FA in male but not female monkeys (ATxSex; p=0.032, one-tailed). Additionally, heritability analyses revealed that variation in UF FA was largely due to non-heritable factors (h^2=0.185, p=0.077).

Conclusions—These results demonstrate a cross-species, male-specific relation between UF microstructure and anxiety, and provide a potential substrate for anxiety-related prefrontal-limbic dysregulation. The heritability analyses point to the importance of environmental influences on UF microstructure, which could be important in mediating the non-heritable components of pathological anxiety. These findings have the potential to guide new treatment strategies for childhood anxiety disorders, and further support the use of nonhuman primates as a translational model to discover mechanisms underlying the development of anxiety.

Keywords

Anxiety; Anxious Temperament; White matter; DTI; Sex Differences; Nonhuman primates

Introduction

Anxiety disorders are among the most prevalent psychiatric disorders, and worldwide it is estimated that ~25% of individuals will experience one or more anxiety disorder in their lifetime (1). The likelihood of developing an anxiety disorder increases through a combination of genetic and environmental factors. For example, having a parent with an anxiety disorder confers increased risk that is likely mediated by heritability and social learning. Estimates for the heritability of anxiety disorders vary between 20–40% (2–6). Anxiety disorders frequently begin in childhood (7) and considerable research has demonstrated the ability to detect an early life anxious phenotype that is associated with a 3- to 4-fold increased risk to develop anxiety and mood disorders (8, 9). This phenotype, termed behavioral inhibition or the related anxious temperament (AT), is evolutionarily conserved across nonhuman primates and humans (10). Understanding the environmental and genetic factors that influence the neural mechanisms underlying AT has the potential to aid in conceptualizing novel early life intervention strategies.

Children with high levels of behavioral inhibition, similar to young monkeys with extreme AT, display heightened behavioral and physiological reactivity to threat (10–13), responses that are thought to be mediated by the amygdala via projections to its downstream targets (14, 15). Nonhuman primate studies demonstrate that increased amygdala metabolism is associated with AT (16–18), a finding that is consistent with studies of individuals with a history of behavioral inhibition as well as individuals suffering from anxiety disorders (19–21). Additionally, in both nonhuman primates and humans, reduced functional coupling between the amygdala and regulatory regions like the prefrontal cortex (PFC) is associated with AT and anxiety disorders (22–25).

The uncinate fasciculus (UF) is of interest, because it is a key white matter structure that is involved in fronto-limbic connectivity (26–28). Research in both adults and children has implicated the UF as a pathway that is altered in anxiety disorders (29–34). Specifically, adults with anxiety disorders display significantly reduced UF fractional anisotropy (FA), a measure of white matter microstructure (29–34). Our previous study in preadolescent

children with anxiety disorders more directly link these findings to pathophysiological mechanisms, because the reduced UF FA cannot be attributed to illness chronicity and/or psychotropic medication exposure (35). Importantly, the reduction in UF FA associated with anxiety disorders appears to be sexually dimorphic, such that it was observed in boys and not girls (35). Further studies elaborating the factors underlying this effect in males will be important in understanding sex-specific pathophysiologies and in deriving novel treatment targets.

The rhesus monkey is ideally suited to uncover mechanisms relevant to human pathological anxiety because of similarities between humans and rhesus monkeys in brain structure and function, and in social and emotional behavior (13, 36, 37). Like humans, the rhesus monkey has a well-developed prefrontal cortex, with similar connectivity between the amygdala and the PFC conveyed by the UF (13). Therefore, we developed and validated a reliable nonhuman primate model of AT focused on understanding the early risk to develop anxiety and other stress related psychopathology. With this model, using a large fully phenotyped multigenerational pedigree, we defined the neural circuit that underlies AT (16, 17, 38). We also found that AT was approximately 29% heritable (17), similar to the heritability observed in human anxiety and anxiety disorders (2–6), and that metabolism in components of the AT circuit are also significantly heritable (17).

We now use this large sample to understand whether the reduction in UF FA observed in boys with anxiety disorders is evolutionarily conserved, and if so, the extent to which it is influenced by heritable and non-heritable factors. More specifically, we explore the hypothesis that AT is related to UF FA, and as in human children, that this effect is sexually dimorphic and selectively occurring in males. In addition to the UF, we explore the heritability of microstructure in other prominent white matter tracts. These data set the stage for mechanistic and proof of concept nonhuman primate studies with the ultimate aim of developing new early life circuit-based interventions.

Methods and Materials

Subjects

Behavioral, endocrine and neuroimaging assessments were performed in 594 young rhesus monkeys (Macaca mulatta), of these subjects 581 (326 males, 255 females) animals had usable diffusion imaging data, and were included in this study. The average age was 1.89 years (0.77 standard deviation), with a range between 0.84 and 4.42 years (Supplementary Figure S1). This age in monkeys is roughly equivalent to pre-pubescent children between 3–12 years old. Imaging data from some of these subjects was previously published (16, 17, 24, 39, 40). All 581 animals were from a large multi-generational pedigree of 1928 (805 males, 1123 females) animals across 9 generations (0^{th}-8^{th}). The relations of the animals for which diffusion imaging data was collected can be traced back 1–8 generations, with most animals being 4^{th}-6^{th} generation (see Supplementary Figure S2). For additional details see our previous publications (16, 17, 41). Procedures were performed using protocols approved by the University of Wisconsin Institutional Animal Care and Use Committee (IACUC).

Behavioral Assessment

Rhesus monkeys were exposed to a human intruder paradigm to assess behavioral and endocrine responses to a mild threat. Blood samples were collected to measure plasma cortisol levels post exposure. To create the composite measure of AT, an average of the z-scores of freezing, inverse cooing and cortisol was computed for each subject. For additional details see the supplement.

Endocrine Assessment

Details of the endocrine assessment are reported in the supplement.

Neuroimaging Assessment

MRI acquisitions—In order to investigate white matter microstructure, magnetic resonance imaging (MRI) scans were collected within 4 months of the NEC-exposure. Data were collected at two imaging locations using GE SIGNA 750 3.0T scanners (General Electrics, Waukesha, WI, USA) and a T/R quadrature extremity coil (Invivo Corp, Gainsville, FL). Details of the scan sequence are reported in the supplement. Briefly, the animals were anesthetized and placed in a stereotactic frame inside the MRI coil. T1-weighted anatomical images were collected in addition to diffusion-weighted images, and their corresponding field map. A change in the scan sequence was implemented midway through the study, and this was accounted for by covarying for this variable in the analyses.

DTI analyses—Details of the DTI analyses are reported in the supplement and processing steps as well as code can be found online (http://www.diffusion-imaging.com). Briefly, white matter microstructure was characterized by using the DWI volumes to calculate the local diffusion tensor in each voxel (Figure 1A). DWI volumes were distortion corrected, tensors were estimated and images were normalized to a population template and warped to our previously published 592 rhesus monkey T1-template (http://www.pnas.org/content/112/29/9118; Supplementary Dataset S01) with a 0.625 mm isotropic resolution. The mean population template in 592-space, created from all subjects, was used for deterministic fiber tractography to delineate tracts of interest (Figure 1B/C). Whole-brain fiber tractography was performed, and white matter pathways were iteratively delineate using anatomically defined waypoints (42, 43) (See Figure 1C for the waypoints used in UF extraction). Tracts extracted included: corpus callosum (CC), cingulum bundle (CING), internal capsule (IC), inferior fronto-occipital fasciculus (IFO), stria terminalis/fornix (STRIA/FX) and UF. Due to limited DTI resolution and close proximity of the STRIA and FX, the two pathways were combined. Since we had no *a priori* hypothesis about left versus right tract differences, the bilateral components of each tract were combined into one average. Multiple diffusion measures beyond FA were estimated to characterize and quantify the microstructural tissue properties of each tract, including mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD).

Heritability Analyses

Heritability was estimated based on pedigree information using Sequential Oligogenic Linkage Analysis Routines (SOLAR)-Eclipse software package (44) (http://www.nitrc.org/

projects/se_linux). Quantitative genetic analysis partitions the observed covariance among related individuals into genetic vs. environmental components. The additive genetic variance, i.e. heritability (h^2) was estimated by employing a maximum likelihood variance decomposition based method. For a pedigree of animals the covariance matrix Ω is given by:

$$\Omega = 2 \cdot \Phi \cdot \sigma_g^2 + I \cdot \sigma_e^2$$

Where Ω is the covariance matrix of the phenotype, Φ is the kinship matrix for the pedigree, σ^2_g is the variance in the trait due to additive genetic effects, I is the identity matrix, and σ^2_e is the variance due to unmeasured random effects, i.e. environmental. Variance parameters are estimated by comparing the covariance due to phenotype matrix with the covariance due to kinship matrix (44). Significance is tested by comparing the model where σ^2_g is constrained to 0, to a model where σ^2_g is estimated. The log likelihood ratio was calculated as twice the difference between these models. Under the null hypothesis, the test statistic is distributed as a 50:50 mixture of a chi-square variate with one degree of freedom and a point mass at zero. Heritability was estimated for each behavior and white matter tract for the full group and within males and females separately. To ensure these traits conform to the assumptions of normality, an inverse normal transformation was applied.

We also tested the difference in heritability between UF and the other extracted pathways. This was accomplished by comparing a model where the two heritability estimates of these pairwise tracts were allowed to vary independently with a model that constrained the heritability estimates to be equal. Furthermore, we estimated the shared heritability between mean tract FA and AT, by running a bivariate heritability analysis, where $\rho = 0$. For more details on the heritability estimation methods see previous publications (17, 44).

Statistical Analyses

All statistical analyses were run with robust linear regression models in order to mitigate the effect of outliers on the results. Sex differences in age, weight, behavior and cortisol were calculated. The significance of the interaction between AT and Sex on tract microstructure was calculated. All analyses controlled for age, sex, site, scanner properties, number of NEC exposures and test order. Next, within-sex analyses were run to determine the relation between AT and tract microstructure within the male and female animals separately. Due to our *a priori* hypothesis on the direction of these effects; a negative relation between UF FA and AT in males but not females, the AT by Sex interaction on UF FA and the within-sex regression between AT and UF FA in males were tested one-tailed. All other statistical tests were two-tailed. For the tracts with no *a priori* predictions we applied a Šidák familywise error correction ($\alpha_{SID} = 1 - (1 - \alpha)^{1/m}$). Where m is the number of tracts, in this case 5. Models were run using the statsmodels package in Python (45).

Heritability analyses were run in SOLAR and controlled for age, age^2, sex, and the age by sex interaction. When the analyses were run within sex, the covariates included age and age^2. SOLAR output included the heritability value (h^2), significance (p), and the standard error.

Results

AT in Female and Male Monkeys

Males and females did not differ in their expression of AT (p = 0.195; Table 1), however, freezing levels tended to be higher in males compared to females ($z(579) = 1.726$, $p = 0.084$; Table 1). Other AT constituents such as coo vocalizations and cortisol levels, as well as weight, did not significantly differ between males and females ($p > 0.28$; Table 1). On average males were slightly younger than the females ($z(579) = -2.197$, $p = 0.024$; Table 1).

Assessment of UF FA in Relation to AT

Using the methods described, we characterized the UF in our population of rhesus monkeys. As can be seen in Figure 2, this tract is highly similar to that we previously characterized in preadolescent children (35). Based on our previous work, we predicted that UF FA would be negatively related to AT in males, but not in females (35). Results demonstrated this predicted effect. Specifically, we found an interaction between AT and Sex on UF FA that was averaged across right and left tracts ($z(570) = -1.861$, $p = 0.032$, one-tailed; Supplementary Table S2; Figure 3). We also analyzed UF FA from each hemisphere individually, which resulted in similar findings (Supplementary Table S3). Similar to our findings in humans, analyses in the male monkeys revealed a significant negative relation between UF FA and AT ($z(317) = -1.794$, $p = 0.037$, one-tailed; Supplementary Table S2, Figure 3). Consistent with the interaction being driven by the males, no significant relation between UF FA and AT was observed in females ($z(246) = 1.288$, $p = 0.198$; two-tailed; Supplementary Table S2, Figure 3). Additionally, there was not a significant main effect for the relation between UF FA and AT across males and females ($p = 0.326$, two-tailed; Supplementary Table S2).

Follow-up analyses examined whether the individual components of AT (freezing, cooing, cortisol) were also related to UF FA. Similar to the UF-AT relation, we found a significant Sex by Freezing interaction ($z(570) = -2.465$, *$p = 0.007$, one-tailed;* Supplementary Table S4), and a significant Sex by Coo vocalizations interaction ($z(570) = 1.826$, *$p = 0.034$, one-tailed;* Supplementary Table S4) on UF FA. However, we did not observe a Sex by Cortisol interaction on UF FA ($z(570) = 0.599$, *$p = 0.275$, one-tailed;* Supplementary Table S4). The anxiety related effects were specific to UF FA, as none of the other tracts or other diffusion measures (MD, AD or RD) demonstrated main effects, or sex interactions (See supplementary Table S2 *and* Table S5). As was observed in the UF, the other tracts are largely homologous to equivalent human tracts (Supplementary Figure S3).

We tested main effects and interactions for Age, Sex and AT on UF FA. While UF FA was significantly negatively affected by age ($z(567) = -2.103$, $p = 0.035$; Supplementary Table S4), the rate of UF FA change did not significantly differ between sexes ($p = 0.618$; Supplementary Table S4). Additionally, there were no significant AT by Sex by Age interactions *($p = 0.413$;* Supplementary Table S4).

Heritability

Because of interest in the genetic and environmental factors that mediate anxiety, we used our large multigenerational pedigree to perform analyses estimating the heritability of AT, its components, and tract microstructure. Our previous work demonstrated AT to be significantly heritable in a larger sample that included the animals in this study (17). We first verified that these findings held across males and females for whom we had DTI data (h^2 = 0.292, $p < 0.001$; Table 2). Because we identified sex differences in the UF-AT relation, we separately examined the heritability of AT in the males and females. We found higher heritability estimates in the females compared to the males, however, the 95% confidence intervals (CI) for these heritability estimates were overlapping (Females: $h^2 = -0.02 – 0.89$ 95% CI; Males: $h^2 = -0.00 – 0.58$ 95% CI; Supplementary Table S6). We also found that AT's components (coo vocalizations, cortisol and freezing) were significantly heritable (p's < 0.025; Supplementary Table S6).

Because of the relation between AT and UF FA, we performed an analysis examining the heritability of UF FA. Across males and females the results indicated low heritability for UF FA (h^2 = 0.185, p = 0.077; Table 2), as well as low heritability when analyzing the males and females separately (Females: h^2 = 0, p = 0.5; Males: h^2 = 0.236, p = 0.12; Table 2). These results indicate that the majority of the variance in UF FA can be explained by non-heritable factors. To determine if this was specific to the UF, or if this was more general of white-matter during this developmental period, we examined the heritability of FA within five additional tracts (CC, CING, IC, IFO, STRIA/FX). Interestingly, in contrast to the UF, FA in all of the other extracted tracts demonstrated significant heritability (p's < 0.005; Table 2). Tests comparing the heritability of UF FA with the other tracts confirmed that UF FA heritability was significantly less than that in the CC (Chi^2 = 5.66, p = 0.017; Supplementary Table S7) and the IFO (Chi^2 = 6.41, p = 0.011; Supplementary Table S7). Even though UF FA was not significantly heritable, we explored the possibility that UF FA would demonstrate co-heritability with AT. Not surprisingly, we found that UF FA was not significantly co-heritable with AT; FA in the other white matter tracts examined was also not co-heritable with AT (Supplementary Table S8 for an overview in the full sample). Consistent with largely non-heritable variation in UF, heritability analyses of other UF diffusivity measures (MD, AD and RD) were not significant (see Supplementary Table S9 for details, as well as data across all the other tracts).

Discussion

Early-life anxious temperament is a risk factor for the later development of anxiety and depressive disorders. Our previous work in young children with anxiety disorders, demonstrated a sex-specific decrease in UF FA in young boys with anxiety disorders, which was not seen in young girls (35). Here, using our well-validated rhesus monkey model of AT, we focused on the relation between AT and white matter microstructure in the UF. The UF is important because it is the major white matter pathway connecting prefrontal with limbic regions and likely plays a role in mediating the transfer of information relevant to adaptive and maladaptive emotion regulation (26, 27). The current results demonstrate a sexually dimorphic relation between UF FA and AT, such that in young male monkeys higher levels

of AT are associated with lower UF FA values. These findings extend our work in anxious young children, and suggest an evolutionarily conserved relationship between UF microstructure and early-life anxiety in boys but not girls (35).

We emphasize that prefrontal-limbic dysregulation is thought to contribute to pathological anxiety in both males and females. The current data point to a potential mechanism in males that does not appear to be present in females. It is conceivable that there are multiple pathways that can result in prefrontal-limbic dysregulation. For example, dysregulation could result from overactivity of the amygdala, decreased input to the amygdala from orbital frontal cortex, or altered function of other prefrontal regions involved in cognition and emotion regulation, such as the dorsolateral prefrontal cortex. While a few studies in the literature have adequate sample sizes to confidently examine sex differences, most studies addressing these issues are under powered. More clearly understanding mechanisms in males and females that underlie prefrontal-amygdala regulation is an important question for future research.

The observed relation between AT and white matter microstructure is specific to UF FA, as no other white matter tracts or other measures of white matter integrity were related to AT. Variation in FA can be attributed to genetic and/or environmental factors. Importantly, in the current study, we found that in the pre-adolescent to early adult age range studied, individual differences in UF FA appear to be largely determined by nonheritable factors. These results point to the importance of early life environmental influences in determining white matter microstructure that is relevant to prefrontal-limbic function as it relates to the expression of AT. While we did not find heritability to be a significant determinant of UF FA at this age, it is possible that heritability could play a greater role as individuals mature. This is supported by observations in human twin studies suggesting low levels of UF FA heritability at 9 year of age that increase into adulthood (46–48).

Regardless of the factors that determine individual differences in UF FA, the FA measurement can reflect variation in fiber organization, axonal density, and/or myelination. All of these features of white matter microstructure can be important in determining the speed, timing and accuracy of neural signals. In relation to our findings which point to the importance of environment in determining variation in UF FA, evidence from animal and human studies suggest that white matter pathways can be dynamically altered in response to learning and other experiences (49–51). Imaging studies in animals and humans demonstrate increased FA associated with learning and skill acquisition (50). At a histological level, animal studies demonstrate activity dependent increases in myelin basic protein (52, 53). It is thought that a fundamental component of activity-dependent changes in white matter occurs via signaling at the “axo-myelinic” synapse, which is the interface between immature and mature oligodendrocytes with the axonal membrane (54).

Our finding of the relation between UF FA and AT in males leads to the question of what mechanisms might play a role in this sexually dimorphic effect. Because heritability can be affected by sex, we examined this possibility. However, we did not find substantial sex differences in the estimated heritability of UF FA. It is also unlikely that adolescent-related influences of sex hormones on brain maturation (55) are relevant to this finding, as our

animals were primarily pre-pubertal. In this regard we note that there were no main effects of sex on UF FA, and no Sex by Age interactions. The lack of heritable effects point to the importance of environmental influences on establishing the male specific relation between UF FA and AT. Because this finding is consistent across humans and nonhuman primates, it is possible that sex-related differences in rearing and/or socialization that are conserved across species could be important. Rhesus monkey mothers have been observed to treat their infant male and female offspring differently. For example, mothers display more embrace and approach behaviors towards their female infants(56, 57), and exhibit more mutual gazing with their male infants(58). Furthermore, studies across human and nonhuman species consistently demonstrate sex-related behavioral differences that are manifested early in life, and it is possible that these differences in behavior affect white matter development. For example, studies in rhesus monkeys have demonstrated sex-related differences in social play in which infant males initiate play more frequently and exhibit more rough- and-tumble play(59), while females engage in more approach-avoidance play (60). It is possible that the sex-related differences that we observed between anxiety and white matter microstructure could in part be due to sex-related behavioral differences occurring during periods of heightened white matter neuroplasticity. It is also important to recognize that rodent studies reveal male-female differences in the regulation of oligodendrocytes(61). Females have a higher turnover rate of oligodendrocytes, as characterized by increased proliferation and increased apoptosis (62, 63). Other data implicates testosterone in facilitating white matter repair in experimentally lesioned animals (64).

While the findings of this study are consistent with findings in children with anxiety disorders, we note the current studies limitations. Although we studied a large sample of rhesus monkeys, the age distribution was predominantly limited to preadolescent animals. Thus, it is possible that the observed effect could be age-related. Additionally, we did not collect data relevant to the early rearing environment which precludes our ability to specifically examine the role of environmental events. Finally, we note that the scanning resolution used in this study may have obscured other effects on white matter microstructure.

Our results in young nonhuman primates provide a cross-species confirmation for a male-specific role of the UF in anxiety-related prefrontal-limbic dysregulation. The findings further support the translational relevance of the nonhuman primate AT model. Since we observed that the majority of UF FA variance is due to non-heritable factors, and research is indicating that myelination is dynamic and activity-dependent throughout life, this opens the door for studying myelin changes in relation to current effective interventions as well as thinking about the development of new treatments. The hypothesis would be that enhanced UF FA would facilitate more efficient communication between the PFC and critical limbic structures resulting in adaptive anxiety regulation. Based on the activity dependent nature of myelin plasticity and the role of the UF FA in prefrontal-limbic regulation, it will be important to assess the extent to which changes in UF FA are associated with individual differences in treatment outcomes. We expect that current interventions thought to work by modifying prefrontal- limbic interactions, such as exposure therapy, cognitive behavioral therapy (CBT), and transcranial magnetic stimulation (TMS), would increase UF FA. By

using UF FA as a dependent measure these therapies could be optimized in relation to their intensity, frequency of administration and length of time over which the intervention is used.

The value of the non-human primate model is to explore the molecular mechanisms that mediate these effects on promoting myelin plasticity. Clues could be provided by assessing differences in UF oligodendrocyte gene regulation between high and low anxious monkeys as they relate to microstructural integrity. Identifying the genes that are the most predictive of UF FA will provide insights into molecular targets that could be leveraged to promote UF FA plasticity. Once identified, nonhuman primate models can be used to examine the extent to which the identified genes are causally related to UF FA and AT, as well as to effective treatment strategies.

It is likely that strategies that selectively activate anxiety regulating neural circuitry between PFC and limbic regions could be of benefit. Additionally, efforts to optimize our treatments such that they promote increases in UF FA will be of value. For example, this could be achieved by intensive training over time in relation to the mastery of anxiety related amygdala responses. While current treatments such as exposure therapy involve the acquisition of mastery techniques, the exact parameters necessary to optimally influence white matter are yet unclear. However, evidence suggests that healthful and stress reducing activities such as aerobic exercise, sleep and environmental enrichment positively impact white matter (50, 65). New treatments could be conceptualized that combine strategies that enhance myelination in tracts, such as the UF, with interventions aimed at activating and “training” key brain regions involved in anxiety regulation that are connected by the UF. For example, we predict that increased UF FA could be achieved by repetitive and consistent exposure to anxiety provoking, limbic activating stimuli, in conjunction with mastery training of induced anxiety. Future studies will be important to establish how these early life male-specific relations between UF FA and AT can inform the development of new sex-specific treatment strategies for anxiety disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by National Institutes of Health grants R01-MH046729, R01-MH081884, P50-MH084051 and P50-MH100031 to NHK; P51-OD011106 and P51-RR000167 to the Wisconsin National Primate Research Center; P30-HD003352 to the Waisman Center. We thank H. van Valkenberg, P.H. Roseboom, M.A. Jesson (University of Wisconsin-Madison), J. Rogers (Baylor College of Medicine), J. Blangero (University of Texas-Rio Grande Valley) and the staffs of the Harlow Center for Biological Psychology, Lane Neuroimaging Laboratory at the HealthEmotions Research Institute, Waisman Laboratory for Brain Imaging and Behavior, and Wisconsin National Primate Research Center.

References

1. Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen H-U (2012): Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. Int J Methods Psychiatr Res. 21: 169–84. [PubMed: 22865617]
2. Hettema JM, Neale MC, Kendler KS (2001): A Review and Meta-Analysis of the Genetic Epidemiology of Anxiety Disorders. Am J Psychiatry. 158: 1568–1578. [PubMed: 11578982]

3. Jardine R, Martin NG, Henderson AS, Rao DC (1984): Genetic covariation between neuroticism and the symptoms of anxiety and depression. Genet Epidemiol. 1: 89–107. [PubMed: 6544237]
4. Martin N, Goodwin G, Fairburn C, Wilson R, Allison D, Cardon LR, Flint J (2000): A population-based study of personality in 34,000 sib-pairs. Twin Res. 3: 310–315. [PubMed: 11463152]
5. Calboli FCF, Tozzi F, Galwey NW, Antoniades A, Mooser V, Preisig M, et al. (2010): A genome-wide association study of neuroticism in a population-based sample. PLoS One. 5. doi: 10.1371/journal.pone.0011504.
6. Bouchard TJ, Loehlin JC (2001): Genes, evolution, and personality. Behav Genet. 31.
7. Kessler RC, Ruscio AM, Shear K, Wittchen H-U (2010): Epidemiology of anxiety disorders. Curr Top Behav Neurosci. 2: 21–35. [PubMed: 21309104]
8. Fox N a, Henderson H a, Marshall PJ, Nichols KE, Ghera MM (2005): Behavioral inhibition: linking biology and behavior within a developmental framework. Annu Rev Psychol. 56: 235–262. [PubMed: 15709935]
9. Clauss J a., Blackford JU (2012): Behavioral inhibition and risk for developing social anxiety disorder: A meta-analytic study. J Am Acad Child Adolesc Psychiatry. 51: 1066–1075. [PubMed: 23021481]
10. Kalin N, Shelton S (1989): Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation. Science (80-). 243: 1718–1721.
11. Kagan J (1997): Temperament and the Reactions to Unfamiliarity. Child Dev. 68: 139–143. [PubMed: 9084130]
12. Craske MG, Rauch SL, Ursano R, Prenoveau J, Pine DS, Zinbarg RE (2009): What is an anxiety disorder? Depress Anxiety. 26.
13. Kalin NH, Shelton SE (2003): Nonhuman Primate Models to Study Anxiety, Emotion Regulation, and Psychopathology. Ann N Y Acad Sci. 1008: 189–200. [PubMed: 14998885]
14. Alheid GF, Heimer L (1988): New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: The striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience. 27: 1–39. [PubMed: 3059226]
15. Bandler R, Shipley MT (1994): Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? Trends Neurosci. 17.
16. Oler J a, Fox AS, Shelton SE, Rogers J, Dyer TD, Davidson RJ, et al. (2010): Amygdalar and hippocampal substrates of anxious temperament differ in their heritability. Nature. 466: 864–8. [PubMed: 20703306]
17. Fox AS, Oler J a., Shackman AJ, Shelton SE, Raveendran M, McKay DR, et al. (2015): Intergenerational neural mediators of early-life anxious temperament. Proc Natl Acad Sci. 201508593.
18. Fox AS, Shelton SE, Oakes TR, Davidson RJ, Kalin NH (2008): Trait-like brain activity during adolescence predicts anxious temperament in primates. PLoS One. 3. doi: 10.1371/journal.pone.0002570.
19. Etkin A, Wager TD (2007): Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry. 164: 1476–1488. [PubMed: 17898336]
20. Engel K, Bandelow B, Gruber O, Wedekind D, Holzschneider K, Mulert C (2011): Neuroimaging in anxiety disorders. Dialogues Clin Neurosci. 13: 453–461. [PubMed: 22275850]
21. Martin EI, Ressler KJ, Binder E, Nemeroff CB (2010): The Neurobiology of Anxiety Disorders: Brain Imaging, Genetics, and Psychoneuroendocrinology. Clin Lab Med. 30.
22. Etkin A, Prater KE, Hoeft F, Menon V, Schatzberg AF (2010): Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. Am J Psychiatry. 167: 54554.
23. Hahn A, Stein P, Windischberger C, Weissenbacher A, Spindelegger C, Moser E, et al. (2011): Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. Neuroimage. 56: 881–889. [PubMed: 21356318]
24. Birn RM, Shackman AJ, Oler JA, Williams LE, McFarlin DR, Rogers GM, et al. (2014): Evolutionarily conserved prefrontal-amygdalar dysfunction in early-life anxiety. Mol Psychiatry. 19: 915–922. [PubMed: 24863147]

25. Monk CS, Telzer EH, Mogg K, Bradley BP, Mai X, Louro HMC, et al. (2008): Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. Arch Gen Psychiatry. 65: 568–576. [PubMed: 18458208]
26. Schmahmann JD, Pandya DN, Wang R, Dai G, Arceuil HED, De Crespigny AJ, et al. (2007): Association fibre pathways of the brain: parallel observations from diffusion spectrum imaging and autoradiography. Brain. 130: 630–53. [PubMed: 17293361]
27. Catani M, Howard RJ, Pajevic S, Jones DK (2002): Virtual in Vivo Interactive Dissection of White Matter Fasciculi in the Human Brain. Neuroimage. 17: 77–94. [PubMed: 12482069]
28. Rudebeck PH, Saunders RC, Prescott AT, Chau LS, Murray E a (2013): Prefrontal mechanisms of behavioral flexibility, emotion regulation and value updating. Nat Neurosci. 16: 1140–5. [PubMed: 23792944]
29. Tromp DPM, Grupe DW, Oathes DJ, McFarlin DR, Hernandez PJ, Kral TR a, et al. (2012): Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. Arch Gen Psychiatry. 69: 925–34. [PubMed: 22945621]
30. Phan KL, Orlichenko A, Boyd E, Angstadt M, Coccaro EF, Liberzon I, Arfanakis K (2009): Preliminary evidence of white matter abnormality in the uncinate fasciculus in generalized social anxiety disorder. Biol Psychiatry. 66: 691–4. [PubMed: 19362707]
31. Baur V, Brühl AB, Herwig U, Eberle T, Rufer M, Delsignore A, et al. (2013): Evidence of frontotemporal structural hypoconnectivity in social anxiety disorder: A quantitative fiber tractography study. Hum Brain Mapp. 00: 437–446.
32. Hettema JM, Kettenmann B, Ahluwalia V, McCarthy C, Kates WR, Schmitt JE, et al. (2012): Pilot multimodal twin imaging study of generalized anxiety disorder. Depress Anxiety. 29: 202–9. [PubMed: 21994092]
33. Baur V, Hanggi J, Rufer M, Delsignore A, Jancke L, Herwig U, Beatrix Brühl A (2011): White matter alterations in social anxiety disorder. J Psychiatr Res. 45: 1366–1372. [PubMed: 21705018]
34. Liao M, Yang F, Zhang Y, He Z, Su L, Li L (2014): White matter abnormalities in adolescents with generalized anxiety disorder: a diffusion tensor imaging study. BMC Psychiatry. 14: 41. [PubMed: 24528558]
35. Tromp DPM, Williams LE, Fox AS, Oler JA, Roseboom PH, Rogers GM, et al. (2019): Altered uncinate fasciculus microstructure in childhood anxiety disorders in boys but not girls. Am J Psychiatry. 176: 208–216. [PubMed: 30654645]
36. Belmonte JCI, Callaway EM, Churchland P, Caddick SJ, Feng G, Homanics GE, et al. (2015): Brains, Genes, and Primates. Neuron. 86: 617–631. [PubMed: 25950631]
37. Oishi K, Huang H, Yoshioka T, Ying SH, Zee DS, Zilles K, et al. (2011): Superficially located white matter structures commonly seen in the human and the macaque brain with diffusion tensor imaging. Brain Connect. 1: 37–47. [PubMed: 22432953]
38. Kalin NH, Shelton SE, Fox AS, Oakes TR, Davidson RJ (2005): Brain regions associated with the expression and contextual regulation of anxiety in primates. Biol Psychiatry. 58: 796–804. [PubMed: 16043132]
39. Adluru N, Zhang H, Fox AS, Shelton SE, Ennis CM, Bartosic AM, et al. (2012): A diffusion tensor brain template for rhesus macaques. Neuroimage. 59: 306–18. [PubMed: 21803162]
40. Fox AS, Shelton SE, Oakes TR, Davidson RJ, Kalin NH (2008): Trait-like brain activity during adolescence predicts anxious temperament in primates. PLoS One. 3: e2570. [PubMed: 18596957]
41. Rogers J, Shelton SE, Shelledy W, Garcia R, Kalin NH (2008): Genetic influences on behavioral inhibition and anxiety in juvenile rhesus macaques. Genes, Brain Behav. 7: 463–469. [PubMed: 18045243]
42. Mori S, Kaufmann WE, Davatzikos C, Stieltjes B, Amodei L, Fredericksen K, et al. (2002): Imaging Cortical Association Tracts in the Human Brain Using Diffusion-Tensor-Based Axonal Tracking. 223: 215–223.
43. Tromp D (2016): DTI Tutorial 3 - Fiber Tractography. doi.org. . doi: 10.15200/winn.146228.88526.
44. Almasy L, Blangero J (1998): Multipoint Quantitative-Trait Linkage Analysis in General Pedigrees. Am J Hum Genet. 62: 1198–1211. [PubMed: 9545414]

45. Seabold S, Perktold J (2010): Statsmodels: econometric and statistical modeling with Python. Proc 9th Python Sci Conf pp 57–61.
46. Brouwer RM, Mandl RCW, Peper JS, van Baal GCM, Kahn RS, Boomsma DI, Hulshoff Pol HE (2010): Heritability of DTI and MTR in nine-year-old children. Neuroimage. 53: 1085–1092. [PubMed: 20298793]
47. Brouwer RM, Mandl RC, Schnack HG, van Soelen IL, van Baal GC, Peper JS, et al. (2012): White matter development in early puberty: a longitudinal volumetric and diffusion tensor imaging twin study. PLoS One. 7: e32316. [PubMed: 22514599]
48. Budisavljevic S, Kawadler JM, Dell'Acqua F, Rijsdijk F V, Kane F, Picchioni M, et al. (2016): Heritability of the limbic networks. Soc Cogn Affect Neurosci. 11: 746–757. [PubMed: 26714573]
49. Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, et al. (2014): Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. Science. 344: 1252304. [PubMed: 24727982]
50. Sampaio-Baptista C, Johansen-Berg H (2017): White Matter Plasticity in the Adult Brain. Neuron. . doi: 10.1016/j.neuron.2017.11.026.
51. Fields RD, Dutta DJ (2019): Treadmilling Model for Plasticity of the Myelin Sheath. Trends Neurosci. . doi: 10.1016/j.tins.2019.04.002.
52. Sampaio-Baptista C, Khrapitchev a a, Foxley S, Schlagheck T, Scholz J, Jbabdi S, et al. (2013): Motor Skill Learning Induces Changes in White Matter Microstructure and Myelination. J Neurosci. 33: 19499–19503. [PubMed: 24336716]
53. Blumenfeld-Katzir T, Pasternak O, Dagan M, Assaf Y (2011): Diffusion MRI of structural brain plasticity induced by a learning and memory task. PLoS One. 6: e20678. [PubMed: 21701690]
54. Micu I, Plemel JR, Caprariello A V, Nave KA, Stys PK (2018): Axo-myelinic neurotransmission: A novel mode of cell signalling in the central nervous system. Nat Rev Neurosci. 19.
55. Perrin JS, Hervé P-Y, Leonard G, Perron M, Pike GB, Pitiot A, et al. (2008): Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. J Neurosci. 28: 9519–9524. [PubMed: 18799683]
56. Mitchell GD (1968): Attachment differences in male and female infant monkeys. Child Dev. 39: 611–620. [PubMed: 4967766]
57. Kulik L, Langos D, Widdig A (2016): Mothers make a difference: Mothers develop weaker bonds with immature sons than daughters. PLoS One. 11 : e0154845. [PubMed: 27191403]
58. Dettmer AM, Kaburu SSK, Byers KL, Murphy AM, Soneson E, Wooddell LJ, Suomi SJ (2016): First-time rhesus monkey mothers, and mothers of sons, preferentially engage in face-to-face interactions with their infants. Am J Primatol. 78: 238–246. [PubMed: 26581804]
59. Brown GR, Dixson AF (2000): The development of behavioural sex differences in infant rhesus macaques (Macaca mulatta). Primates. 41: 63–77. [PubMed: 30545192]
60. Meaney MJ, Stewart J, Beatty WW (1985): Sex Differences in Social Play: The Socialization of Sex Roles. Adv Study Behav. (Vol. 15), pp 2–58.
61. Swamydas M, Bessert D, Skoff R (2009): Sexual dimorphism of oligodendrocytes is mediated by differential regulation of signaling pathways. J Neurosci Res. . doi: 10.1002/jnr.21943.
62. Cerghet M, Skoff RP, Swamydas M, Bessert D (2009): Sexual dimorphism in the white matter of rodents. J Neurol Sci. . doi: 10.1016/j.jns.2009.06.039.
63. Cerghet M (2006): Proliferation and Death of Oligodendrocytes and Myelin Proteins Are Differentially Regulated in Male and Female Rodents. J Neurosci. . doi: 10.1523/JNEUROSCI.2219-05.2006.
64. Hussain R, Ghoumari AM, Bielecki B, Steibel J, Boehm N, Liere P, et al. (2013): The neural androgen receptor: A therapeutic target for myelin repair in chronic demyelination. Brain. . doi: 10.1093/brain/aws284.
65. Hughes EG, Orthmann-Murphy JL, Langseth AJ, Bergles DE (2018): Myelin remodeling through experience-dependent oligodendrogenesis in the adult somatosensory cortex. Nat Neurosci. 21: 696–706. [PubMed: 29556025]

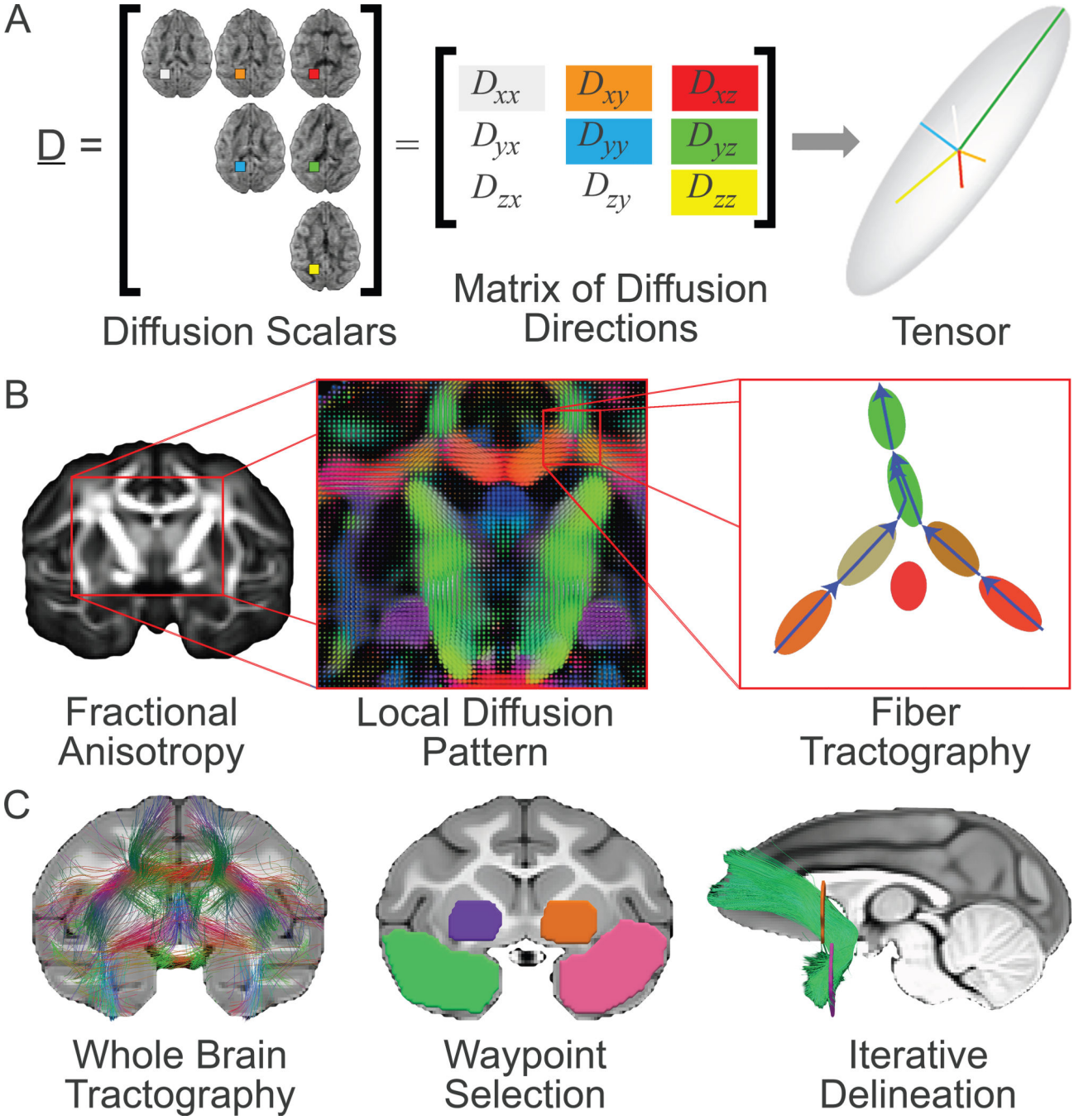


Figure 1. DTI methods.

Overview of methods used for diffusion tensor imaging (DTI) processing and analysis. A) The scanner collects a scalar image of water diffusion throughout the brain at multiple non-collinear directions. Combining the amount of water diffusion with the applied matrix of diffusion directions provides a diffusion tensor for each voxel in the brain. B) Local brain microstructure is quantified by using diffusion measures such as fractional anisotropy (FA), where bright regions represent highly anisotropic tensors and dark regions indicate more isotropic tensors, indicating an underlying microstructure with high and low levels of white

matter fibers, respectively. Next, fiber tractography can be applied to follow the direction of each tensor to get an estimate of the underlying white matter anatomy. C) After running fiber tractography throughout the whole brain, waypoint selection can be used to delineate fiber tracts of interest using anatomically defined waypoints. Iterative delineation must be used to minimize the inclusion of spurious tracts.

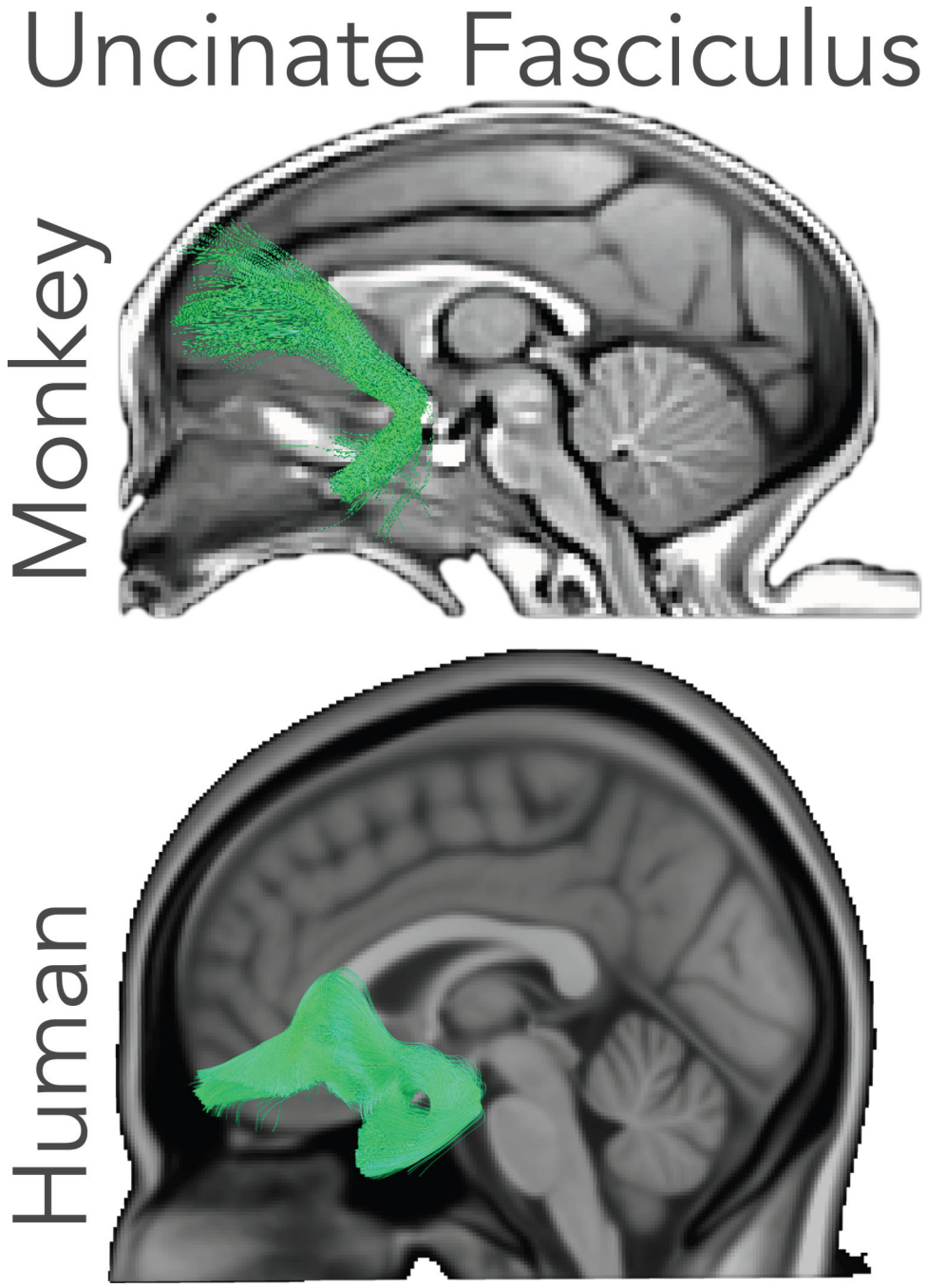


Figure 2. Comparison of UF across species.

Depicted is a comparison of the Uncinate Fasciculus (UF) in humans and monkeys. This qualitative comparison indicates largely evolutionarily conserved white matter architecture. The human image is modified from a previously published image in our study of preadolescent children with anxiety disorders(35).

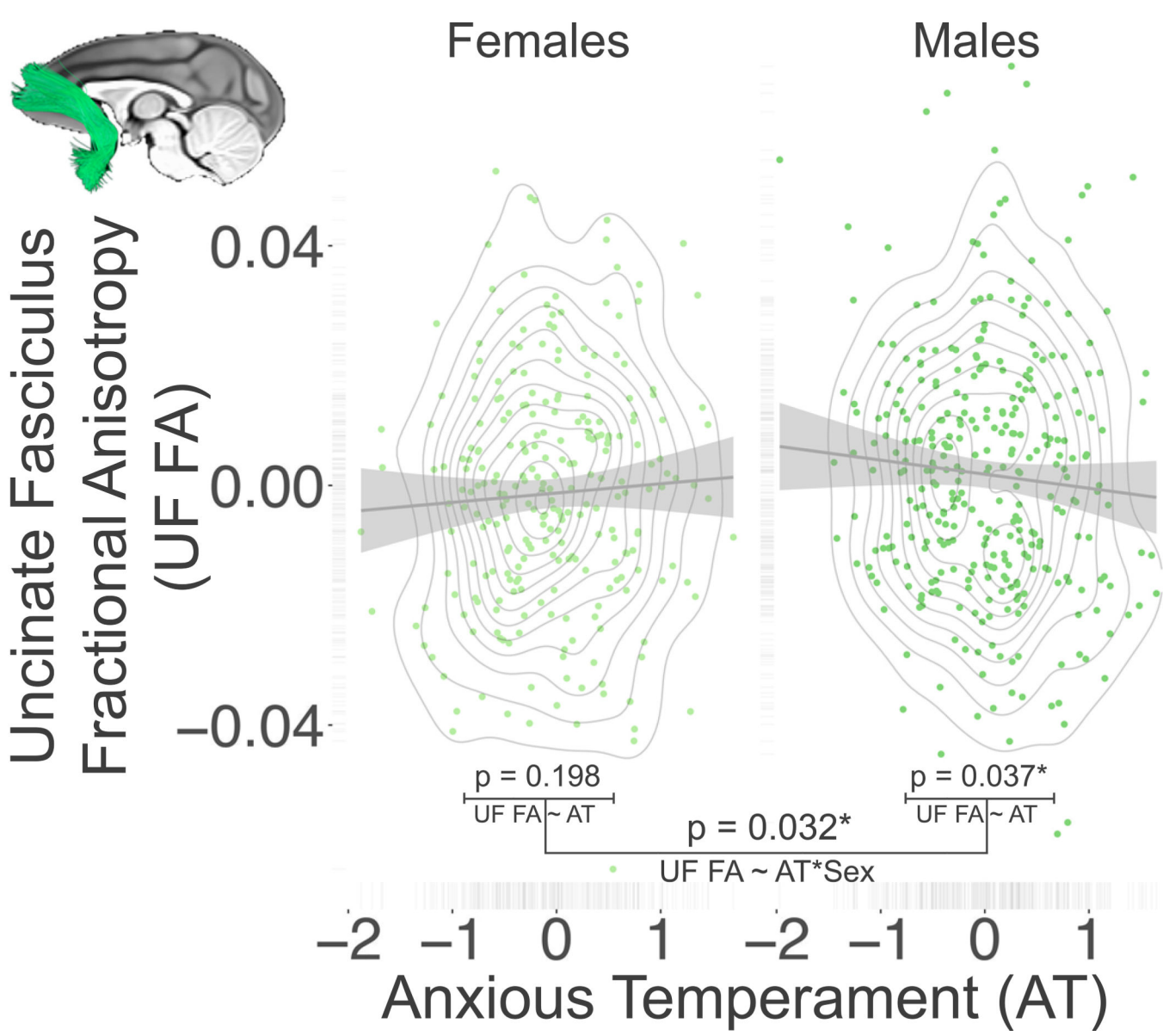


Figure 3. UF FA and AT.

When testing the interaction between anxious temperament (AT) and Sex on uncinate fasciculus (UF) fractional anisotropy (FA), results indicate a one-tailed significant interaction (p = 0.032, one-tailed), such that males (right; dark green) display a negative relation between AT and UF FA (p = 0.037, one-tailed), but females (left; light green) do not (p = 0.198, two-tailed). The scatterplots display linear regression lines with confidence intervals, density estimation contours, and rug plots of the marginal distributions for AT and UF FA values. Plotted values for UF FA are residualized for the covariates.

Table 1.

Demographic information.

Demographic, behavior and cortisol means (± standard deviation) and significance of sex differences are displayed for female and male rhesus monkeys.

	Females	Males	*p* -values
Sample size	255	326	n.a.
Age (years); mean (SD)	1.95 (0.82)	1.80 (0.70)	**0.028** *
Weight (kg); mean (SD)	3.20 (1.19)	3.18 (1.08)	0.528
AT; mean (SD)	−0.05 (0.66)	0.02 (0.66)	0.195
Cortisol (μg/dl); mean (SD)	0.26 (17.61)	1.80 (16.24)	0.286
Coo Vocalizations; mean (SD)	2.25 (2.91)	2.23 (2.70)	0.883
Freezing; mean (SD)	1.41 (1.43)	1.57 (1.42)	0.084

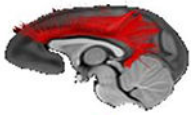
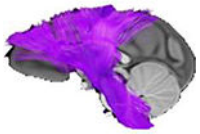
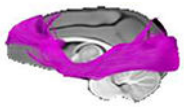
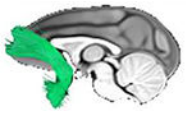
* indicates significance at $p < 0.05$. Statistics for AT and its components used transformed and residualized data.

Abbreviations: Anxious Temperament (AT).

Table 2.

Heritability analyses of FA.

Heritability (mean H^2, standard error and p-values) are presented here for FA in each white matter tract, for all subjects, and for females and males separately.

Heritability		All Subjects (n = 581)		Females (n = 255)		Males (n = 326)	
		H^2 (SE)	*p* -values	H^2 (SE)	*p* -values	H^2 (SE)	*p* -values
Tract FA:							
CC		0.57 (0.121)	**<0.001** **	0.504 (0.207)	**0.004** **	0.735 (0.179)	**<0.001** **
CING		0.346 (0.112)	**<0.001** **	0.345 (0.183)	**0.017** *	0.325 (0.142)	**0.002** **
IC		0.227 (0.082)	**<0.001** **	0.364 (0.179)	**0.007** **	0.284 (0.133)	**0.004** **
IFO		0.496 (0.116)	**<0.001** **	0.443 (0.198)	**0.007** **	0.554 (0.178)	**<0.001** **
STRIA/FX		0.322 (0.129)	**0.001** **	0.418 (0.234)	**0.021** *	0.187 (0.151)	0.073
UF		0.185 (0.142)	0.077	0	0.500	0.236 (0.22)	0.120

* indicates uncorrected significance at $p < 0.05$

** indicates sidak corrected significance at $p < 0.0085$.

Abbreviations: Corpus Callosum (CC), Cingulum bundle (CING), Fractional Anisotropy (FA), Internal Capsule (IC), Inferior Fronto-Occipital fasciculus (IFO), Standard Error (SE), Stria Terminalis & Fornix (STRIA/FX), Uncinate Fasciculus (UF).