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White matter microstructure varies with post-traumatic stress severity following medical trauma.

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Abstract

The prefrontal cortex, amygdala, hippocampus, and hypothalamus are important components of the neural network that mediates the healthy learning, expression, and regulation of emotion. These brain regions are connected by white matter pathways that include the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis. Individuals with trauma and stress-related disorders show dysfunction of the cognitive-affective processes supported by the brain regions these white matter tracts connect. Therefore, variability in the microstructure of these white matter pathways may play an important role in the cognitive-affective dysfunction related to post-traumatic stress disorder. Thus, the current study used diffusion weighted imaging to assess the white matter microstructure of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis acutely (< 1 month) following trauma. Further, we assessed both acute (i.e., < 1 month) and subacute (i.e., 3 months post-trauma) post-traumatic stress symptom severity. White matter microstructure (assessed < 1 month post-trauma) of the uncinate fasciculus and fornix/stria terminalis varied with acute post-traumatic stress severity (assessed < 1 month post-trauma). Further, white matter microstructure (assessed < 1 month post-trauma) of the cingulum bundle and fornix/stria terminalis varied with subacute post-traumatic stress severity (assessed 3 months post-trauma). The current results suggest white matter architecture of the prefrontal cortex – amygdala network plays an important role in the development of trauma and stress-related disorders.

Keywords

Tractography; Acute stress; PTSD; Cingulum bundle; Uncinate fasciculus; Stria terminalis

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1. Introduction

Acute Stress Disorder (ASD) and Post-Traumatic Stress Disorder (PTSD) are characterized by dysfunction of cognitive-affective processes that are supported by a network of brain regions that includes the prefrontal cortex (PFC), amygdala, hippocampus, and hypothalamus (Delgado et al. 2008; Denny et al. 2014; Dretsch et al. 2016; LaBar et al. 1998; Milad et al. 2008, 2009; Milad, Wright, et al. 2007; Phillips and LeDoux 1992). These regions are interconnected by axonal projections that form distinct white matter tracts which include the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis (Mori et al. 2008; Wakana et al. 2004). Therefore, variability in the white matter microstructure of these tracts may play an important role in susceptibility to trauma and stress-related disorders. However, limited prior human neuroimaging research has investigated white matter microstructure acutely (i.e., < 1 month) following trauma (Chen et al. 2013; Hu et al. 2016). Thus, the relationship between post-traumatic stress and white matter tracts that connect brain regions such as the PFC, amygdala, hippocampus, and hypothalamus remains unclear. Understanding the impact that white matter microstructure has on post-traumatic stress severity may elucidate neurobiological processes that underlie susceptibility to post-traumatic stress.

Brain regions that include the PFC, amygdala, hippocampus, and hypothalamus interact to support cognitive-affective processes that are important for healthy emotion learning, expression, and regulation (Critchley et al. 2000; Knight et al. 2009; Milad, Quirk, et al. 2007; Milad, Wright, et al. 2007). Specifically, the dorsomedial PFC supports detection and monitoring processes that are important for learned fear (Choi et al. 2012; Etkin et al. 2011; Knight et al. 1999; Mobbs et al. 2010; Shackman et al. 2011). In turn, the ventromedial PFC supports direct regulation of the emotional response via connections to the amygdala (Berretta et al. 2005; Likhtik 2005; Motzkin et al. 2015; Pitman et al. 2012; Urry 2006). The amygdala supports fear learning and expression of the peripheral emotional response via projections to the hypothalamus (Helmstetter 1992; Helmstetter and Bellgowan 1993; Knight et al. 2005; LeDoux et al. 1988, 1990). Hypothalamic activity is also modulated by input from the hippocampal formation (Jacobson and Sapolsky 1991; Meibach and Siegel 1977), which is important for emotional learning and memory processes (Kjelstrup et al. 2002; Knight et al. 2004; Milad, Wright, et al. 2007; Phillips and LeDoux 1992). Specifically, the hypothalamus controls the peripheral expression of emotion via projections to the autonomic nervous system (Jansen et al. 1995). The hypothalamus further influences the peripheral expression of emotion via hormone release as part of the hypothalamic-pituitary-adrenal (HPA) axis (Buijs and Van Eden 2000; Timpl et al. 1998). Notably, prior research has demonstrated the emotional learning, expression, and regulation processes the PFC, amygdala, hippocampus, and hypothalamus support are disrupted in PTSD (Dretsch et al. 2016; Fani et al. 2012; Jovanovic et al. 2010; Milad et al. 2008).

The brain regions that support emotion expression and regulation are connected via white matter tracts that include the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis (Mori et al. 2008; Wakana et al. 2004). Specifically, the dorsal segment of the cingulum bundle interconnects medial regions of the cerebrum, including the dorsomedial and ventromedial PFC. Another tract, the uncinate fasciculus, extends from the PFC to the

temporal pole and connects the ventromedial PFC to the amygdala. Further, the amygdala projects to the hypothalamus via the stria terminalis. The stria terminalis lies adjacent to the fornix which connects the hippocampus to the hypothalamus. Given the role of these white matter tracts in emotional processes, the microstructure of these tracts (i.e., cingulum bundle, uncinate fasciculus, and fornix/stria terminalis) may be important for understanding cognitive-affective disruptions in PTSD. In humans, white matter microstructure can be assessed through reconstruction of diffusion weighted images (Basser et al. 2000; Catani et al. 2002; Hagmann et al. 2006; Mori et al. 1999). Prior research using diffusion weighted imaging (DWI) methods has identified alterations in the microstructure of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis in individuals with PTSD (Fani et al. 2015, 2016; Kennis et al. 2015; Koch et al. 2017; Olson et al. 2017; Sanjuan et al. 2013). This prior DWI work indicates that the white matter microstructure of the cingulum bundle and uncinate fasciculus varies inversely with post-traumatic stress severity (Fani et al. 2015, 2016; Koch et al. 2017; Olson et al. 2017; Sanjuan et al. 2013). Further, changes in microstructure of the fornix/stria terminalis appear to play a role in the success of PTSD treatment. Specifically, reductions in fornix/stria terminalis microstructure metrics are associated with symptom improvement (Kennis et al. 2015). These findings suggest microstructure of the white matter tracts that connect the PFC and subcortical brain structures may play a significant role in PTSD symptom expression. Further, recent reports have suggested variability in the microstructure of these tracts may play an important role in the development and expression of PTSD (Chen et al. 2013; Hu et al. 2016). Taken together, prior research suggests white matter tracts connecting the PFC, amygdala, hippocampus, and hypothalamus may underlie PTSD symptom development and expression.

Although changes in the microstructure of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis have been implicated in PTSD, it is unclear whether these changes develop acutely after trauma exposure or are a product of chronic PTSD. Prior research has largely examined white matter microstructure in chronic PTSD patients. Findings from this prior research have been mixed, with some studies demonstrating significant differences, and others finding no difference, in white matter microstructure between chronic PTSD patients and healthy controls (Dretsch et al. 2017; Sanjuan et al. 2013). Importantly, changes in white matter microstructure may be delayed following trauma exposure given these changes often occur over weeks to months (Boekel et al. 2017; Piervincenzi et al. 2017). Therefore, changes in white matter microstructure may be more common in chronic PTSD patients. However, limited research has investigated white matter microstructure acutely following trauma exposure. Therefore, whether differences in white matter microstructure develop acutely following trauma exposure remains unclear. Thus, investigations of white matter microstructure acutely following trauma are necessary to better understand the neural substrates that mediate PTSD development.

In the present study, we assessed white matter microstructure of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis and investigated the relationship between acutely assessed white matter microstructure, trauma, and PTSD symptom severity. First, we investigated white matter differences within the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis related to trauma exposure. We compared the microstructure of these white matter tracts between recently Trauma-Exposed (TE) and Non-Trauma-Exposed

(NTE) participants. White matter microstructure was assessed acutely (i.e., < 1 month) following trauma in TE participants. We hypothesized that the TE group would show reduced white matter microstructure within the cingulum bundle and uncinate fasciculus, as well as greater fornix/stria terminalis microstructure compared to the NTE group. Second, we investigated the relationship between post-traumatic stress symptom severity and white matter microstructure in TE participants. Specifically, we investigated the relationship between acute (i.e., assessed at < 1 month) and subacute (i.e., assessed at 3 months) post-traumatic stress severity and microstructure of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis in recently TE participants. We hypothesized that the white matter microstructure of our *a priori* tracts of interest would vary with acute (i.e., < 1 month) post-traumatic stress severity. Specifically, we anticipated white matter microstructure of the cingulum bundle and uncinate fasciculus would show an inverse relationship with post-traumatic stress severity. In addition, we hypothesized a positive relationship would be observed between fornix/stria terminalis microstructure and post-traumatic stress severity. Further, we anticipated that the white matter microstructure of these tracts would vary with subacute (i.e., at 3 months) post-traumatic stress severity. The present study identifies white matter correlates of acute and subacute post-traumatic stress severity, and sheds new light on the role of white matter pathways that may mediate susceptibility to post-traumatic stress.

2. Methods

Participants:

The present study used a prospective design in which imaging data were collected prior to assessment of subacute post-traumatic symptom severity. Specifically, TE participants completed diffusion weighted imaging during an initial assessment of acute post-traumatic stress symptom severity (< 1 month). Participants were later interviewed a second time at 3 months post-trauma and their subacute post-traumatic stress symptom severity was assessed. Volunteers in the present study participated in prior published work that investigated the relationship between brain glutamate levels and acute post-traumatic stress symptoms (Harnett et al. 2017). Twenty-one TE participants were recruited for the present study. Two TE participants terminated participation prior to scanning and three participants' data were lost to equipment malfunction. Thus, 16 TE participants (Age: $M = 28.88$, $SD = 8.03$; 12 Male) were included in the present analysis. TE participants were recruited from the University of Alabama at Birmingham's Acute Trauma Care Unit, Trauma Burn Nursing Unit, Trauma Burn Intensive Care Unit, and Emergency Department, and were scanned within 30 days of trauma exposure ($M = 21.88$ days, $SD = 4.70$ days). TE participants had experienced 1) a physical injury requiring a visit to a trauma unit/Emergency Department and 2) exposure to a traumatic event identified on the Posttraumatic Diagnostic Scale (PDS) (e.g., accident, fire, etc.) within the last 30 days (Table S1). Nineteen NTE control participants (Age: $M = 24.26$, $SD = 3.43$; 14 Male) were recruited from the Birmingham-Metropolitan Area. All participants were screened for exclusion criteria prior to the study. Exclusion criteria included: history of blood or circulation disorders (e.g., anemia or sickle-cell), diabetes, brain or spinal abnormalities, pregnancy, previous or current head injury (e.g., traumatic brain injury (TBI), concussion, or loss of consciousness), or report of previous/current diagnosis of mental illness that began prior to the traumatic event. All

participants provided written informed consent as approved by the University of Alabama at Birmingham Institutional Review Board.

Posttraumatic Diagnostic Scale:

Participants completed the PDS (Foa 1995; Foa et al. 1987). The PDS is a self-report assessment of stress disorder symptoms (e.g., avoidance, arousal, and re-experiencing symptoms) consisting of 49 items. NTE control participants were screened for any exposure to a traumatic event (no NTE participants endorsed experiencing a traumatic event within their lifetime), and all subsequent items were scored as 0 (i.e., participants were scored as having no post-traumatic symptoms as there was no trauma). Participants completed the PDS a second time 3 months after trauma exposure. Scores from the PDS were compared between the TE and NTE groups to confirm differences in post-traumatic stress symptoms (Table 1).

Psychosocial Risk Factor Survey:

Participants completed the Psychosocial Risk Factor Survey (PRFS), a 70-item self-report questionnaire that assessed depression, anxiety, anger/hostility, social isolation, and emotional guardedness (Eichenauer et al. 2010). Each item was scored using a 5-point Likert scale ranging from 0 (Strongly Agree) to 4 (Strongly Disagree). Total and subscale PRFS scores were compared between TE and NTE groups (Table 1).

Wechsler Test of Adult Reading:

All participants completed the Wechsler Test of Adult Reading (WTAR). The WTAR was administered and scored according to established guidelines (Venegas and Clark 2011). The WTAR consists of 50 words that participants pronounced aloud, and the accuracy of their pronunciation was scored to provide a measure of intellectual function (Venegas and Clark 2011). Participants read the list of 50 words until they either declared the words too difficult, could not pronounce seven consecutive words correctly, or completed the list. One TE participant left the study before completing the WTAR. WTAR raw scores were calculated based on the number of words participants correctly pronounced (one point for each correctly pronounced word). Raw scores were then converted to a standard score based on an age-adjusted representative sample of United States examinees. The standard score for each participant was converted to a WAIS-III Full Scale Intelligence Quotient (FSIQ). FSIQ scores were compared between the TE and NTE groups (Table 1).

Diffusion Weighted Imaging:

Diffusion imaging was completed on a 3 Tesla Siemens Allegra scanner. Sixty diffusion weighted volumes (TR=4600 ms, TE=79 ms, FOV=24 cm, voxel = 2.5 mm × 2.5 mm × 2.5 mm, b-value=1000 s/mm², sixty directions) were acquired. Data were analyzed using DSI Studio (February 2016 build) (Yeh et al. 2013). Motion and eddy current effects were reduced via registration to a b0 image as implemented in DSI Studio. Q-space diffeomorphic reconstruction (QSDR) (sampling length ratio = 1.25 mm, orientation distribution function tessellation = 8-fold, 5 fibers resolved) was used to reconstruct the data in Montreal Neurologic Institute standard space via trilinear interpolation with resampling to 2 mm × 2

mm $\times$ 2 mm grid spacing (Yeh and Tseng 2011). QSDR reconstructs a spin distribution function to obtain quantitative anisotropy (QA) values and QA-guided tractography may better characterize white matter pathways compared to fractional anisotropy (FA)-guided tractography (Yeh et al. 2013). Further, generalized FA (GFA) can be derived from the spin distribution function in QSDR and GFA is comparable to FA (Cohen-Adad et al., 2008; Tuch, 2004). Thus, QA-guided tractography may allow for better resolution of white matter tracts while providing metrics that are comparable to prior FA-based reports on PTSD white matter microstructure.

Tractography was completed using a deterministic fiber tracking algorithm (Yeh et al. 2013). Fiber tracts of interest were created using a combination of the Johns Hopkins University White Matter Atlas (JHU) and the Harvard-Oxford Cortical/Subcortical Atlas (HOCS) within DSI studio, as well as custom regions of avoidance (e.g., slabs through the sagittal midline to separate right and left hemispheres) to constrain the streamlines. Specifically, individual tracts of the uncinate fasciculus, cingulum bundle, and fornix/stria terminalis were reconstructed (1500 streamlines per tract, minimum/maximum length = 30/300 mm, turning angle = 40 degrees, QA threshold = 0.2, step size = 1) resulting in six tracts bilaterally (Figure 1; see supplementary material for additional detail on tract reconstruction).

Statistical Analyses:

Statistical analyses were completed using SPSS Statistics Version 25 (IBM Corp., Armonk, NY). Demographic and self-report data were compared between the TE and NTE groups using independent-samples t-tests and Chi-square tests as appropriate. GFA values for the white matter tracts of interest were compared between the TE and NTE groups using repeated-measures analyses of covariance (RM-ANCOVAs). An RM-ANCOVA was chosen to account for the inclusion of within-subjects and between-subjects factors and continuous covariates. The first of these RM-ANCOVAs included a within-subjects factor of hemisphere (left and right), a within-subjects factor of tract (cingulum bundle and uncinate fasciculus), a between-subjects factor of group (TE and NTE), and a covariate for age. The fornix/stria terminalis did not reconstruct for three NTE participants and could not be included in the first RM-ANCOVA due to differences in subject numbers available for analysis. Therefore, a second RM-ANCOVA was completed that included a within-subjects factor of hemisphere (left and right fornix/stria terminalis), a between-subjects factor of group (TE and NTE), and a covariate for age. We also completed multiple linear regression analyses to assess the unique relationship GFA of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis had with acute and subacute PDS scores within the TE group. The multiple linear regressions included GFA for each tract and participant age as predictors, and either acute or subacute PDS scores as the dependent variable (two separate models). If a significant relationship was found between the GFA of a white matter tract and PDS scores, exploratory follow-up partial correlations were completed to determine the relationship between the individual tract and PDS subscale scores while covarying for the other tracts and age. We also assessed changes in PDS scores over time using correlation analysis, paired samples t-tests, and one-sample t-tests to assess individual variability in PDS scores. Further, an additional RM-ANCOVA was completed to determine if the relationship between GFA of individual tracts varied with the change in PDS scores between the acute and subacute

phases. The RM-ANCOVA included a within-subjects factor of time (PDS scores at the acute and subacute phases) and continuous covariates for GFA of the white matter tracts of interest and age. Finally, we completed an RM-ANCOVA to determine if GFA of the white matter tracts of interest varied with PRFS scores. The RM-ANCOVA included a within-subjects factor for tract (cingulum bundle, uncinate fasciculus, and fornix/stria terminalis), a between-subjects factor for group (TE and NTE), and a covariates for age and total PRFS score. A nominal significance threshold of $p < 0.05$ was used.

3. Results

3.1 Participant demographics

Chi-square and independent samples t-tests were completed to compare demographics of the TE and NTE groups. There were no significant differences in race, gender, handedness, or FSIQ between the TE and NTE groups (Table 1). A significant difference in age was observed [$t(33) = 2.27$, $p = 0.030$, $d = 0.75$] between the TE and NTE groups. Therefore, age was included as a covariate in group comparisons. As expected, TE participants reported significantly greater post-traumatic stress symptoms than the NTE participants (Table 1). Specifically, TE participants reported greater re-experiencing, avoidance, arousal, and total scores on the PDS compared to NTE participants.

3.2 Group-level analyses of white matter microstructure

RM-ANCOVAs were completed to assess differences in GFA between TE and NTE groups. The RM-ANCOVAs revealed no significant differences in GFA between TE and NTE groups within the cingulum bundle, uncinate fasciculus, or fornix/stria terminalis (all $p > 0.05$). These results indicate white matter pathways were not significantly different between TE and NTE groups.

3.3 Relationship between white matter microstructure and post-traumatic stress

Multiple regression analyses were completed to assess the relationship between white matter microstructure and acute post-traumatic stress severity within the TE group. Given that the prior RM-ANCOVAs did not reveal an effect of hemisphere, the GFA for each tract was averaged over hemisphere for further analyses. Regressors for GFA of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis were included as well as a covariate for age. The multiple regression analysis revealed significant relationships between GFA of the uncinate fasciculus [$t(11) = -2.29$, $\beta = -0.57$, $p = 0.043$] and fornix/stria terminalis [$t(11) = 2.50$, $\beta = 0.62$, $p = 0.030$] and total PDS scores (assessed < 1 month post-trauma). A significant effect of age was also observed [$t(11) = -3.64$, $\beta = -0.72$, $p = 0.004$]. Exploratory follow-up partial correlations investigated the relationship between PDS subscale scores and GFA of the uncinate fasciculus and fornix/stria terminalis. GFA of the uncinate fasciculus varied negatively with re-experiencing scores ($r = -0.59$, $p = 0.034$), but not avoidance ($r = -0.49$, $p = 0.087$) or arousal ($r = -0.38$, $p = 0.202$) scores. In contrast, GFA of the fornix/stria terminalis varied positively with arousal scores ($r = 0.76$, $p = 0.003$), but not re-experiencing ($r = 0.50$, $p = 0.085$) or avoidance ($r = 0.33$, $p = 0.278$) scores. These results suggest the white matter microstructure of the uncinate fasciculus and fornix/stria terminalis are related to the severity of acute (i.e., < 1 month) post-traumatic stress symptoms.

Next, we assessed whether white matter microstructure (assessed acutely) varied with subacute (i.e., PDS assessment at 3 months post-trauma) post-traumatic stress severity. A second multiple regression was completed to investigate the relationship between GFA of the white matter tracts of interest and PDS scores at 3 months post-trauma. Three TE participants did not complete the 3 month assessment and were therefore not included in analyses assessing the relationship between white matter microstructure and post-traumatic stress severity. Thus, 13 participants were included in these analyses. The total PDS scores during the acute phase ($M = 14.15$, $SD = 9.96$) and 3 month follow-up ($M = 12.15$, $SD = 12.69$) did not significantly differ ($p > 0.05$, $d = 0.21$). PDS scores during the acute phase and 3 month follow-up were positively correlated, suggesting symptoms were generally stable for the TE group across time [$r = 0.66$, $p < 0.05$].

To assess individual variability in PDS scores, the absolute value of the difference in PDS scores from 1 to 3 months was included in a one-sample t-test against 0 (i.e., no change in PDS score). The analysis revealed significant within-subject variability in PDS scores ($M = 7.38$, $SD = 6.31$) over time [$t(12) = 4.22$, $p < 0.01$, $d = 1.17$]. Specifically, PDS scores did not change for two participants (15%), increased for five participants (38%), and decreased for six participants (46%). These findings indicate that while the severity of post-traumatic stress experienced by the TE group as a whole did not change, there was significant individual variability in post-traumatic stress severity over time.

The multiple regression analysis revealed a significant effect of GFA of the fornix/stria terminalis [$t(8) = 4.57$, $\beta = 1.19$, $p = 0.002$] and cingulum bundle [$t(8) = -2.44$, $\beta = -0.59$, $p = 0.041$] on three month PDS scores. A significant effect of age was also observed [$t(8) = -3.73$, $\beta = -0.65$, $p = 0.006$]. Exploratory follow-up partial correlation analyses were completed to assess the relationship between PDS subscale scores and GFA within the fornix/stria terminalis and cingulum bundle. The partial correlation analyses revealed that GFA within the fornix/stria terminalis varied positively with re-experiencing [$r = 0.78$, $p = 0.008$], avoidance [$r = 0.79$, $p = 0.006$], and arousal [$r = 0.78$, $p = 0.008$] scores. In contrast, GFA of the cingulum bundle varied negatively with arousal ($r = -0.66$, $p = 0.038$), but did not vary with re-experiencing ($r = -0.37$, $p = 0.298$) or avoidance ($r = -0.60$, $p = 0.070$) scores. Taken together, these data suggest white matter microstructure of the fornix/stria terminalis and cingulum bundle are related to the subacute expression of post-traumatic stress symptoms.

The current results suggested microstructure of white matter tracts varied with both acute (uncinate fasciculus and fornix/stria terminalis) and subacute (cingulum bundle and fornix/stria terminalis) post-traumatic stress symptom expression. However, there was substantial variability in participant PDS scores between 1 and 3 months. Specifically, some participants post-traumatic stress severity improved while others' declined. However, it was unclear whether white matter microstructure was related to the change in post-traumatic stress severity over time. Thus, an additional RM-ANCOVA was completed to assess the relationship between white matter GFA and total PDS scores over time. A trend-level interaction was observed for the time by GFA effect within the fornix/stria terminalis [$F(1,8) = 5.06$, partial $\eta^2 = 0.39$, $p = 0.055$]. These data suggest GFA of the fornix/stria terminalis

may be more strongly related to the severity of subacute, rather than acute, post-traumatic stress symptoms.

Results from the multiple linear regression analyses suggested that the microstructure of white matter tracts varied with both acute (uncinate fasciculus and fornix/stria terminalis) and subacute (cingulum bundle and fornix/stria terminalis) post-traumatic stress symptom severity. We next sought to determine whether white matter microstructure varied uniquely with post-traumatic stress severity or if white matter microstructure was also related to variations in cognitive-affective processes more broadly. Therefore, an RM-ANCOVA was completed to assess the relationship between PRFS scores and white matter GFA, as well as interactions between group and PRFS scores on white matter GFA. The RM-ANCOVA did not reveal a significant main effect of PRFS scores [$F(1,27) = 3.12$, partial $\eta^2 = 0.10$, $p = 0.089$], a significant tract by PRFS score interaction [$F(2,54) = 0.91$, partial $\eta^2 = 0.03$, $p = 0.411$], or a significant tract by group by PRFS score interaction [$F(2,54) = 2.67$, partial $\eta^2 = 0.09$, $p = 0.079$]. However, a tract by group interaction did near significance [$F(2,54) = 3.08$, partial $\eta^2 = 0.10$, $p = 0.054$]. These results suggest that white matter microstructure does not vary with cognitive-affective dysfunction more broadly.

4. Discussion

White matter pathways such as the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis connect brain regions that support important cognitive-affective processes (e.g., emotional learning, expression, and regulation). Disruption of cognitive-affective function is a hallmark of trauma-induced stress disorders such as ASD and PTSD. Therefore, the microstructure of white matter pathways may play an important role in the development of post-traumatic stress disorders (e.g., ASD and PTSD). Thus, the objective of the current study was to investigate the white matter pathways (assessed acutely following trauma exposure) that connect brain regions that support cognitive-affective processes to determine the relationship between white matter microstructure and post-traumatic stress severity. The white matter microstructure of the uncinate fasciculus varied negatively, while microstructure of the fornix/stria terminalis varied positively, with acute post-traumatic stress severity. In addition, the white matter microstructure of the fornix/stria terminalis varied positively, while microstructure of the cingulum bundle varied negatively, with subacute post-traumatic stress severity. Taken together, the present study demonstrates individual differences in the neuroarchitecture of white matter pathways that underlie the expression of post-traumatic stress symptoms during the acute and subacute phases following trauma exposure.

The present study investigated white matter microstructure within an understudied population. Specifically, DWI was completed with individuals acutely following medical trauma exposure. Prior human neuroimaging research has largely focused on individuals with chronic PTSD (Davenport et al. 2015; Sanjuan et al. 2013). For example, participants in much of the prior research completed neuroimaging months or even years after the traumatic event. In the current study, participants were scanned within 30 days of the traumatic event. Therefore, the present results are not a consequence of long-term changes that may co-occur with chronic PTSD. Further, prior research has often studied PTSD in veteran populations

that have suffered a concomitant TBI (Davenport et al. 2015; Hoge et al. 2008). However, participants with a TBI, head trauma, or loss of consciousness were excluded from the present study to avoid confounding factors that may be associated with post-concussive syndrome, white matter shearing, or other brain damage following a traumatic event (Meythaler et al. 2001; Seo et al. 2012). Therefore, the present results are unlikely the result of the physical force of the traumatic event. Instead, the current data are most likely related to either preexisting structural architecture or variations in white matter that develop acutely following trauma exposure.

The present study focused on white matter tracts that connect key brain regions implicated in PTSD. Specifically, we focused on brain regions that included the PFC, amygdala, hippocampus, and hypothalamus as these brain regions are critical for healthy emotional function. Importantly, healthy emotional function is mediated by a series of neurochemical signals within and between these brain regions. Prior research has demonstrated PTSD is associated with dysfunctional neurochemical signaling (Pitman et al., 2012). For example, endocannabinoid systems partially regulate stress-related changes in emotional function via modulation of glutamatergic and GABAergic activity within the PFC and amygdala, and also impact HPA axis reactivity (Hill et al. 2010, 2018; Ramikie et al. 2014; Ruehle et al. 2012). Traumatic stress may disrupt endocannabinoid systems leading to dysfunctional PFC-amygdala activity and the hyperarousal that has been linked to PTSD. Further, PTSD is associated with enhanced noradrenergic activity that underlies the hyperarousal and re-experiencing symptoms patients experience (Liberzon, Abelson, et al. 1999; Pitman et al. 2012; Southwick et al. 1993). Dysfunction of glucocorticoid receptor activity is also associated with PTSD (Pitman et al. 2012; Yehuda et al. 1991), and may be linked to learning, memory, and HPA axis dysfunction (Kohda et al. 2007; Liberzon, Lopez, et al. 1999; Sandi 2011). Thus, alterations in the neurobiology of the PFC, amygdala, hippocampus, and hypothalamus are related to PTSD and may underlie individual differences in white matter microstructure. Therefore, understanding individual differences in the white matter tracts that connect these regions is important for elucidating the mechanisms that underlie variability in PTSD symptom expression.

Our analyses revealed a negative relationship between white matter microstructure of the uncinate fasciculus and acute post-traumatic stress severity (Figure 2). Specifically, greater GFA of the uncinate fasciculus was associated with lower total PDS scores during the acute phase (i.e., < 1 month) following trauma. The uncinate fasciculus connects the ventromedial PFC and the amygdala. The ventromedial PFC supports emotion regulation processes that are disrupted in individuals with PTSD (Milad et al. 2009; Milad, Wright, et al. 2007). Specifically, the ventromedial PFC regulates the amygdala-mediated expression of the peripheral emotional response (Milad and Quirk 2002; Motzkin et al. 2015; Urry 2006). Therefore, stronger connections between the ventromedial PFC and amygdala may result in greater inhibition of the emotional response and more effective emotion regulation. The present study demonstrates that acute post-traumatic stress severity decreases as white matter microstructure of the uncinate fasciculus increases. These data suggest that stronger structural connections between the ventromedial PFC and the amygdala may support greater emotion regulation of acute post-traumatic stress symptoms. Further, the present findings are consistent with recent reports indicating there may be changes in the white matter

microstructure of the uncinate fasciculus as a function of post-traumatic stress (Costanzo et al. 2016; Koch et al. 2017; Olson et al. 2017). Taken together, regulation of emotional function, acutely following trauma, may be dependent on the strength of white matter connections (i.e., the uncinate fasciculus) between the ventromedial PFC and amygdala.

While the microstructure of the uncinate fasciculus was negatively related to acute (< 1 month post-trauma) post-traumatic stress severity (Figure 2), microstructure of the fornix/stria terminalis was positively related to symptom expression at both the acute and subacute (i.e., at 3 months) assessments (Figure 3). Specifically, as GFA (measured acutely) within bilateral fornix/stria terminalis increased, individuals reported greater acute and subacute post-traumatic stress symptom severity. The fornix and stria terminalis connect the hippocampus and amygdala to the hypothalamus. Projections from the hypothalamus are important for autonomic nervous system and HPA axis responses to stress (Buijs and Van Eden 2000). Notably, the amygdala and hippocampus appear to modulate hypothalamic activity and therefore play an important role in the expression of the peripheral emotional response (Herman et al. 1989; LeDoux et al. 1988; Petrovich et al. 2001). In the present study, we observed a positive relationship between the white matter of the fornix/stria terminalis (assessed within 1 month post-trauma) and both acute and subacute post-traumatic stress symptoms. These data suggest that stronger connections from the amygdala and hippocampus to the hypothalamus play an important role in expression of post-traumatic stress. Taken together, amygdala and hippocampal projections to the hypothalamus (i.e., fornix/stria terminalis) may underlie expression and maintenance of PTSD symptoms.

In the present study, white matter microstructure of the cingulum bundle varied negatively with subacute post-traumatic stress severity. The dorsal segment of the cingulum bundle runs through the cingulate gyrus, connecting the ventromedial and dorsomedial PFC and other posterior brain regions (e.g., posterior cingulate cortex). Prior research has observed differences in brain activity and neurometabolite concentrations between PTSD patients and controls within these brain regions (Etkin and Wager 2007; Harnett et al. 2017; Meyerhoff et al. 2014; Milad et al. 2009; Shin et al. 2001, 2011; Yang et al. 2015). Further, prior DWI research has observed decreased microstructure of the dorsal cingulum bundle in PTSD patients (Jenkins et al. 2016; Sanjuan et al. 2013), and an increase in white matter microstructure following treatment for PTSD (Kennis et al. 2015). In the present study, we observed a negative relationship between post-traumatic stress severity and white matter of the dorsal cingulum bundle. Taken together, these findings suggest the microstructure of the dorsal cingulum may play an important role in the long-term development of PTSD.

Our findings suggest a dissociation between acute and 3 month post-traumatic stress assessments and acutely measured microstructure of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis. The white matter of the cingulum bundle and uncinate fasciculus varied negatively with post-traumatic stress, while the white matter of the fornix/stria terminalis varied positively with post-traumatic stress. One possibility for this dissociation in white matter and post-traumatic stress is that these tracts may subserve separate neural processes that are linked to post-traumatic stress symptom expression. Specifically, the cingulum bundle and uncinate fasciculus may be important for regulation of post-traumatic stress symptoms, while the fornix/stria terminalis may be important for

symptom expression and maintenance. Further, these systems may interact such that individuals with weaker connections (i.e., the cingulum bundle and uncinate fasciculus) between the brain regions (e.g., dorsomedial PFC and ventromedial PFC) that support top-down, cognitive control processes fail to inhibit emotional responses related to post-traumatic stress. The subsequent failure to regulate emotion may be exacerbated by the stronger connections (i.e., fornix/stria terminalis) between the amygdala and hippocampus to the hypothalamus, which may lead to long-term emotion dysfunction (i.e., PTSD symptoms). In our analysis, the fornix/stria terminalis appeared to show a stronger relationship with subacute than acute post-traumatic stress severity. We speculate that the transition from acute to chronic post-traumatic stress may rely on a shift from neural circuitry that supports emotional regulation processes to other predominant circuits supporting emotional expression (i.e., the fornix/stria terminalis). However, the current study is unable to disentangle the potential interactions between the neural circuits that mediate post-traumatic stress symptom severity and time. In the present study, neuroimaging was only conducted during the acute phase and not at the 3-month follow-up. Therefore, although the present results suggest acutely assessed white matter varies predictably with post-traumatic stress, it remains unclear whether the microstructure of these white matter tracts change over time or if one system may influence another directly. Future work should seek to assess neurobiological changes longitudinally after trauma to determine potential alterations in these neural systems over time.

Another important consideration in the present study concerns whether white matter microstructure variation is a cause or an effect of post-traumatic stress. Specifically, white matter microstructure may predispose an individual to PTSD (i.e., a trait marker) or may only covary with PTSD (i.e., a state marker). If white matter microstructure is a trait for PTSD, then it may be possible to predict an individual's likelihood of developing PTSD prior to a traumatic event. Specifically, it may be possible to determine susceptibility to *potential* post-traumatic stress disorder in non-traumatized individuals. In the present study, we did not observe a significant difference in white matter microstructure between TE and NTE groups. These data may suggest that traumatic experiences do not lead to acute (i.e., < 1 month) changes in the white matter architecture that supports emotional processes. Thus, if traumatic experiences do not induce acute changes in white matter, then the white matter variation may preexist (i.e., the same before the trauma as after). However, the TE participants in the present study were included on the basis of trauma, not based on the severity of their acute post-traumatic stress symptoms. Thus, it is also possible that the heterogeneity of the TE group masked potential differences between the TE and NTE groups. Future research should thus compare NTE participants with symptomatic and non-symptomatic TE individuals to better determine the impact of trauma-exposure on white matter architecture.

In the present study, TE and NTE participants differed in feelings of anger/hostility. Specifically, TE participants reported greater feelings of anger/hostility as measured by the PRFS compared to the NTE group. One potential reason for the increased anger/hostility score in the TE group is the exposure to the trauma itself. The anger/hostility subscale assesses how easily or frequently an individual feels frustrated or angry, or inappropriately displays anger or hostility towards others (Eichenauer et al. 2010). Exposure to traumatic or

highly stressful events can produce acute disruptions in emotional processes and may lead to increased volatility and anger (Amstadter and Vernon 2008; Jakupcak and Tull 2005). Given the NTE participants did not experience a traumatic event, the difference in anger/hostility between the two groups may be a result of trauma exposure. It is also possible that, instead of an acute result of trauma exposure, the increased anger/hostility in the TE group is a preexisting factor. Individuals who have higher general feelings of anger are more likely to develop and express PTSD symptoms, and may be more likely to experience trauma (Lommen et al. 2014; Orth and Wieland 2006). Further, anger as a preexisting risk factor for trauma may be related to other risk factors such as risk-taking behavior and impulsivity (Lerner and Keltner 2001; Redeker et al. 1995). Thus, it is difficult to determine the specific reasons the TE group showed increased anger/hostility compared to the NTE group in the present study. Speculatively, it is likely a combination of both pretraumatic and peritraumatic factors, however the exact extent of either cannot be determined in the present study. Nevertheless, future research on the mechanisms underlying PTSD susceptibility should assess participants' feelings of anger as this may be important for determining PTSD risk.

The findings of the present study should be interpreted with several considerations. First, the current study was not able to separate white matter tracts specific to the fornix and stria terminalis due to the voxel resolution acquired during diffusion imaging. Prior reports have successfully separated the fornix/stria terminalis both *in-vivo* and *ex-vivo*, however accurate delineation of the stria terminalis from the fornix requires high-resolution information not available in the present study (Avery et al. 2014; Mori et al. 2017; Mori and Aggarwal 2014). Therefore, it is unclear whether the relationship between white matter microstructure and post-traumatic stress severity is specific to the fornix, stria terminalis, or both. Second, a relatively small sample of TE individuals were included in the present analyses. Usable DWI data was available for only 16 participants, with 13 participants completing follow-up assessments. The restricted sample size may affect the generalizability of the present findings. Third, pain severity was not considered in the present study. Pain sensation is partially supported by activity within the dorsomedial PFC and prior research suggests the cingulum bundle may play a role in pain nociception (Atlas et al. 2014; Fuchs et al. 1996; Shackman et al. 2011). Thus, future research may need to more fully consider pain severity in the relationship between white matter microstructure and PTSD symptom expression. Finally, imaging was limited to one session preventing a longitudinal assessment of potential white matter changes related to trauma-exposure. Future research should assess white matter microstructure both acutely and over time to determine whether neurobiological changes coincide with the progression of PTSD symptoms.

The present study investigated white matter microstructure of the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis acutely following a medical trauma. The microstructure of the uncinate fasciculus varied inversely with acute post-traumatic stress symptom severity. Further, microstructure of the cingulum bundle varied inversely with subacute post-traumatic stress severity. Finally, the white matter of the fornix/stria terminalis varied positively with both acute and subacute post-traumatic stress symptom severity. The present findings elucidate the structural architecture underlying stress disorder susceptibility

and suggest white matter pathways of the PFC, amygdala, hippocampus, and hypothalamus may play a key role in the expression of acute and subacute post-traumatic stress symptoms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

White matter tracts of interest. Tractography was completed for each subject to generate the cingulum bundle, uncinate fasciculus, and fornix/stria terminalis white matter pathways.

Figure 2.

Uncinate fasciculus and post-traumatic stress. A negative relationship was observed between white matter microstructure of the uncinate fasciculus and acute post-traumatic stress severity indexed by the Posttraumatic Diagnostic Scale (PDS). The graph depicts the partial correlation between generalized fractional anisotropy (GFA) of the uncinate fasciculus and PDS scores at < 1 month post-trauma for each participant (dots). The solid black line reflects the line of best fit.

Figure 3.

Fornix/stria terminalis and post-traumatic stress. A positive relationship was observed between white matter microstructure of the fornix/stria terminalis (assessed < 1 month post-trauma) and both acute (assessed < 1 month) and subacute (3 month assessment) post-traumatic stress severity indexed by the Posttraumatic Diagnostic Scale (PDS). Graphs depict the partial correlation between generalized fractional anisotropy (GFA) of the fornix/stria terminalis and acute (left) and subacute (right) PDS scores for each participant (dots). The solid black line reflects the line of best fit.

Figure 4.

Cingulum bundle and post-traumatic stress. A negative relationship was observed between white matter microstructure of the cingulum bundle (assessed < 1 month post-trauma) and subacute (3 month assessment) post-traumatic stress severity indexed by the Posttraumatic Diagnostic Scale (PDS). The graph depicts the partial correlation between generalized fractional anisotropy (GFA) of the cingulum bundle and subacute PDS scores for each participant (dots). The solid black line reflects the line of best fit.

Table 1.**Participant Demographics**

	TE	NTE	p-value
<u>Age</u>	28.88 (8.03)	24.26 (3.43)	0.030
<u>Race</u>	9EA/7AA	10EA/9AA	0.830
<u>Sex</u>	12M/4F	14M/5F	0.929
<u>Handedness</u>	14R/2L	17R/2L	0.855
<u>FSIQ</u>	92.33 (13.65)	98.74 (14.87)	0.206
<u>PDS Total Severity (1 month)</u>	13.19 (9.41)	0	< 0.001
└ Re-experiencing Severity	3.94 (2.98)	0	< 0.001
└ Avoidance Severity	4.13 (4.13)	0	0.001
└ Arousal Severity	5.13 (3.50)	0	< 0.001
<u>PDS Total Severity (3 month)</u>	12.15 (12.69)	.	.
└ Re-experiencing Severity	3.77 (4.15)	.	.
└ Avoidance Severity	3.46 (4.48)	.	.
└ Arousal Severity	4.92 (5.35)	.	.
<u>PRFS Total Score</u>	111.25 (30.50)	97.89 (27.73)	0.184
└ Depression	17.38 (9.58)	16.26 (11.04)	0.755
└ Anxiety	20.63 (12.39)	14.21 (7.47)	0.083
└ Anger/Hostility	28.13 (6.83)	21.21 (8.69)	0.015
└ Social Isolation	18.31 (8.68)	19.89 (8.82)	0.598
└ Emotional Guardedness	26.81 (3.21)	26.32 (4.80)	0.727

Note: FSIQ = Full Scale Intelligence Quotient; PDS = Posttraumatic Diagnostic Scale; PRFS = Psychosocial Risk Factor Survey; EA = European-American; AA = African-American; M = Male; F = Female. Values for Trauma-Exposed (TE) and Non-Trauma-Exposed (NTE) groups are presented as: Mean (Standard Deviation).