Abnormal Prefrontal Development in Pediatric Posttraumatic Stress Disorder: A Longitudinal Structural and Functional Magnetic Resonance Imaging Study

Supplemental Information

Supplemental Methods and Materials

Participant Attrition

Within the PTSD group, nine youth (female, n=6; male, n=3) did not complete the follow-up visit due to loss of contact (n=4), declined return (n=3), moved away (n=1), and pregnancy (n=1). When comparing the attrition and retention groups, there were no significant differences in total PTSD symptom severity or sex. The attrition group was marginally older than the retention group at initial scan (t=1.89, p=0.06).

Supplemental Results

Targeted Analyses Exploring Lack of Hippocampal GMV Abnormalities

Based upon our prior age-related hippocampal effects in the baseline sample of this study (1), we expected to find longitudinal abnormalities. However, perhaps due to power issues, we were unable to detect any significant group or longitudinal differences in GMV in the hippocampus. To explore this, we conducted an ROI analysis using the anterior hippocampus cluster identified in our previous work on the baseline sample (1). We then ran a group by time model, covaried for age at baseline, sex, total intracranial volume, and subject as a random effect. No significant group (t=0.71, p=0.48) or group by time (t=0.255, p=0.80; Figure 3) relationships with hippocampal GMV were identified. Next, given evidence that SSRI exposure influences hippocampal volume (2,3), we conducted analyses to assess whether interim SSRI use modulated

hippocampal development over time in youth with PTSD. A separate group by time model was run where youth with PTSD were grouped based on the presence (n=6) or absence (n=16) of SSRI use. Here, we observed a significant relationship between SSRI use during study enrollment and time ($t=2.25$, $p<0.05$; Figure S3). Interim SSRI users showed significantly increasing hippocampal GMV over time, while non-SSRI exposed youth with PTSD and TD youth did not.

Resting-State Prefrontal Connectivity: Seed-Based Analyses

Group main effects in intrinsic connectivity are summarized in Supplemental Table S1. Multivariate regressions within the PTSD youth with the six symptom domains described above (covaried for age at baseline, sex, and TIV) revealed significant negative relationships between dlPFC-hippocampus connectivity and three symptom measures: avoidance symptoms (PTSD-RI subscore C; $t=-2.09$, $p=0.05$), depression symptoms (MFQ; $t=-2.13$, $p=0.04$), and anxiety symptoms (SCARED; $t=-2.38$, $p=0.02$) (Supplemental Figure S2).

In order to localize the clusters identified in the RSFC analyses, overlap analyses used the amygdala subnuclei and hippocampal masks from the SPM Anatomy Toolbox available within SPM12 (Wellcome Department of Imaging Neuroscience, London, UK; (4)). Analyses reveal 100% overlap between the extracted vmPFC-amygdala cluster and the basolateral amygdala anatomical mask, and 100% overlap between the extracted dlPFC-hippocampus and vlPFC-hippocampus cluster with the anterior hippocampus anatomical mask.

Post Hoc Analyses

Post hoc analyses of extracted VBM and resting-state functional connectivity cluster data with clinical and demographic variables were conducted within RStudio. All VBM group main effects and longitudinal group x scan results across analyses remained significant when covaried for IQ, Tanner stage, trauma load, age at index trauma, previous clinical diagnosis of depressive

or anxiety disorder, previous psychotropic medication use, and history of therapy (right vmPFC group main effect: t=-3.06, p=0.004; right vlPFC group main effect: t=-4.16, p<0.001; left vlPFC group main effect: t=-4.03, p<0.001; right precentral gyrus group main effect: t=-4.05, p<0.001; PCC group main effect: t=-3.36, p=0.002; dlPFC group x time effect: t=3.70, p<0.001). Further, age at index trauma was a significant positive predictor of dlPFC GMV (t=2.42, p=0.02). Longitudinal effects in resting-state functional connectivity also remained significant when adjusted for the above variables (vmPFC-left amygdala group x scan effect: t=-3.22, p=0.003; vlPFC-left hippocampus group x scan effect: t=-3.88, p<0.001; dlPFC-left hippocampus group x scan effect: t=-2.86, p=0.007).

Supplemental Tables

Table S1 – Participant Characteristics of VBM and RSFC Analyses

VBM Participant Characteristics

	TD (n=20, F=14)		PTSD (n=22, F=15)	
	Time 1	*Time 2*	*Time 1*	*Time 2*
Age (years)	13.75 (2.5)	15.01 (2.57)	13.58 (2.76)	14.77 (2.77)
Age range	10.34-17.99	11.47-19.31	8.07-17.99	9.17-19.07
IQ (average)	108.6	108.85	101.62	99.59
Tanner Stage	3.15	3.7	2.77	3.64
MFQ	3.05	3.95	24	21.23
SCARED	9.2	8.5	35.18	27
PTSD-RI	-	-	49.9	36.27

RSFC Participant Characteristics

	TD (n=19, F=15)		PTSD (n=23, F=17)	
	Time 1	*Time 2*	*Time 1*	*Time 2*
Age (years)	14.32 (2.38)	15.58 (2.42)	14.42 (2.82)	15.99 (3.15)
Age range	10.48 - 17.99	11.55 - 19.31	8.07 - 18.8	9.17 - 23.4
IQ (average)	110.32	110.32	101.23	100.48
Tanner Stage	3.42	3.89	3.11	4.04
MFQ	-	-	25.43	21.43
SCARED	-	-	34.65	28.17
PTSD-RI	-	-	49.17	36.91

The PTSD Reaction Index, MFQ, and SCARED represent the youth report scores. The CAPS-CA score was not obtained for the first five PTSD participants. Numbers in parentheses represent the standard deviation.

Abbreviations: MFQ, Mood and Feelings Questionnaire; SCARED, Screen for Child Anxiety Related Mood Disorders; CAPS-CA, Clinician-Administered PTSD Scale Child and Adolescent version; PTSD-RI, PTSD Reaction Index.

Table S2 - Summary of Whole-Brain RSFC Longitudinal Analyses

Contrast	Mask	Seed	Target	Laterality	Peak Z	k	x	y	z
Group Main Effects	WB	L dlPFC	Cerebellum	R	5.5	2699	-10	84	-46
	WB	L dlPFC	Precuneus	R	4.78	2075	-16	80	54
	WB	Precentral	Cuneus	B	5.91	738	0	90	4
	WB	Precentral	Cerebellum	R	4.71	723	-40	68	-50
	WB	R vmPFC	Precuneus	R	4.43	1696	-16	70	26

Clusters shown survived whole-brain cluster correction (corrected < 0.05). Peak Coordinates (x, y, z) are reported based on the MNI atlas in the LPI orientation. All analyses included age at baseline, sex, and subject as a random effect.

Abbreviations: dlPFC, dorsolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex.

Supplemental Figures

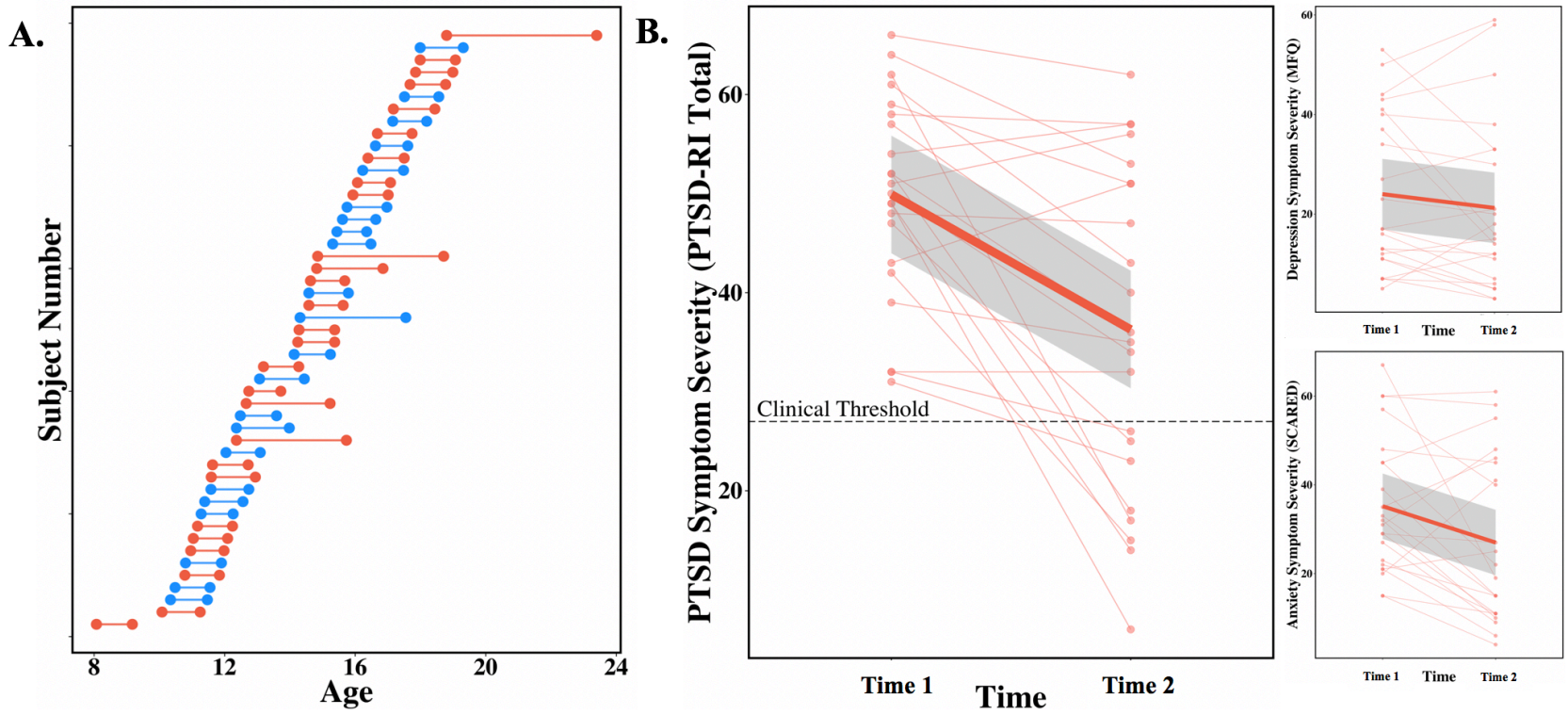

Figure S1: ***Longitudinal subject plot and symptom severity development.*** (A) Longitudinal subject plot by age. Each line represents one subject, and each dot represents the age at scan date (PTSD=red, TD=blue). (B) On average within the PTSD group, PSTD, depression, and anxiety symptoms decreased between baseline and follow-up. Symptom severity was measured using the PTSD-RI total score, MFQ, and SCARED.
Abbreviations: PTSD, posttraumatic stress disorder; TD, typically developing; PTSD-RI, UCLA PTSD Reaction Index; MFQ, Mood and Feelings Questionnaire; SCARED, Screen for Child Anxiety Related Disorders.

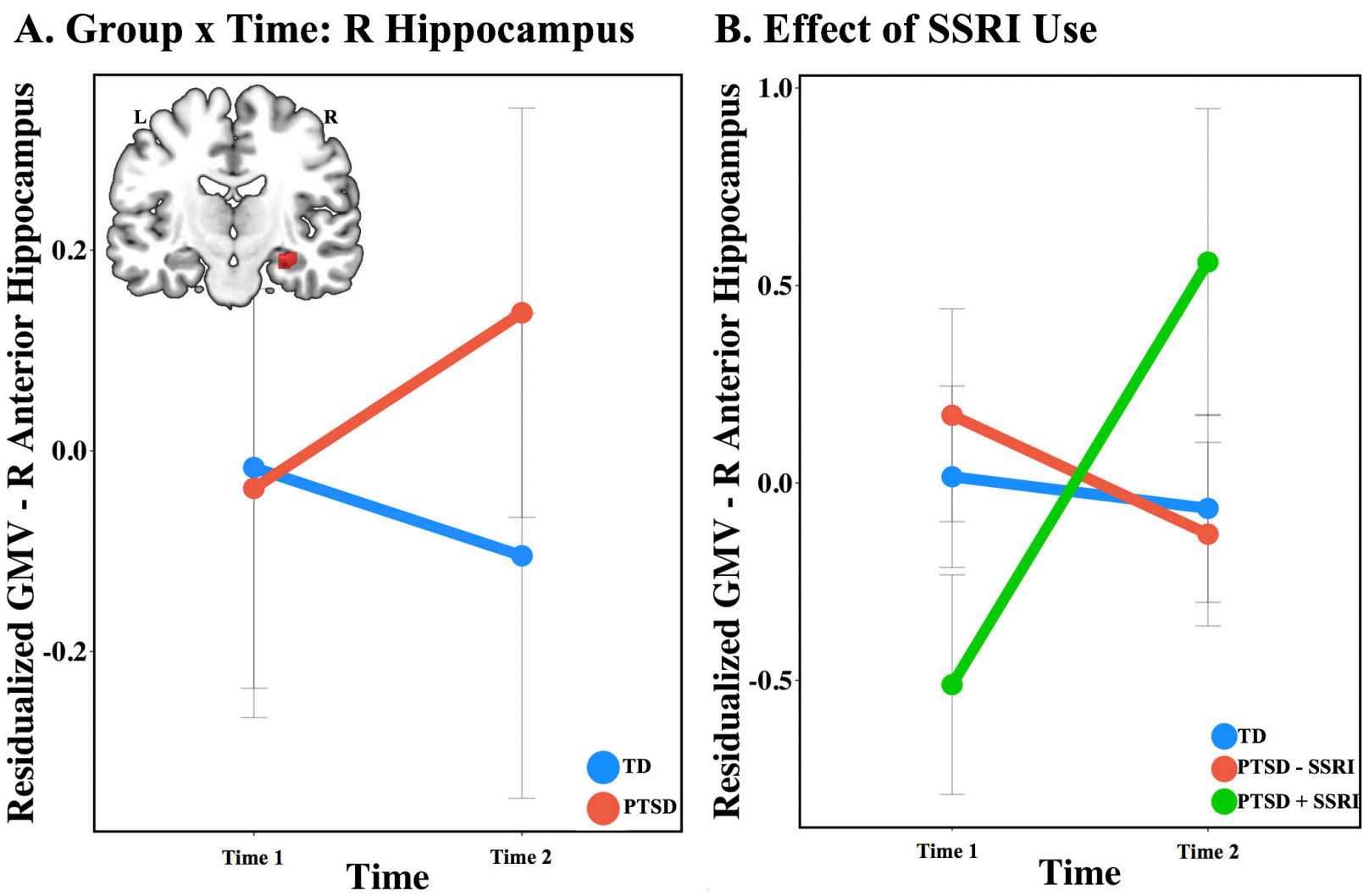

Figure S2: ***Lack of longitudinal hippocampal structural abnormalities in youth with PTSD.*** (A) There was no significant interaction between GMV in the right anterior hippocampus and scan, however youth with PTSD seem to exhibit longitudinal increases in GMV while TD youth show normative longitudinal decreases in right anterior hippocampus GMV. PTSD = red, TD = blue. (B) Further exploration revealed a significant SSRI use X scan interaction in the right anterior hippocampus GMV, covaried for age at baseline, sex, and total intracranial volume ($p<0.05$). TD youth and youth with PTSD that have not taken an SSRI during the course of the study (n=16) showed typical decreases in hippocampal GMV, while youth with PTSD that had taken an SSRI during the course of the study (n=6) showed abnormal longitudinal increases. TD = blue, PTSD-SSRI= red, PTSD+SSRI = green.
Abbreviations: PTSD, posttraumatic stress disorder; GMV, gray matter volume; TD, typically developing; SSRI, selective serotonin-reuptake inhibitor.

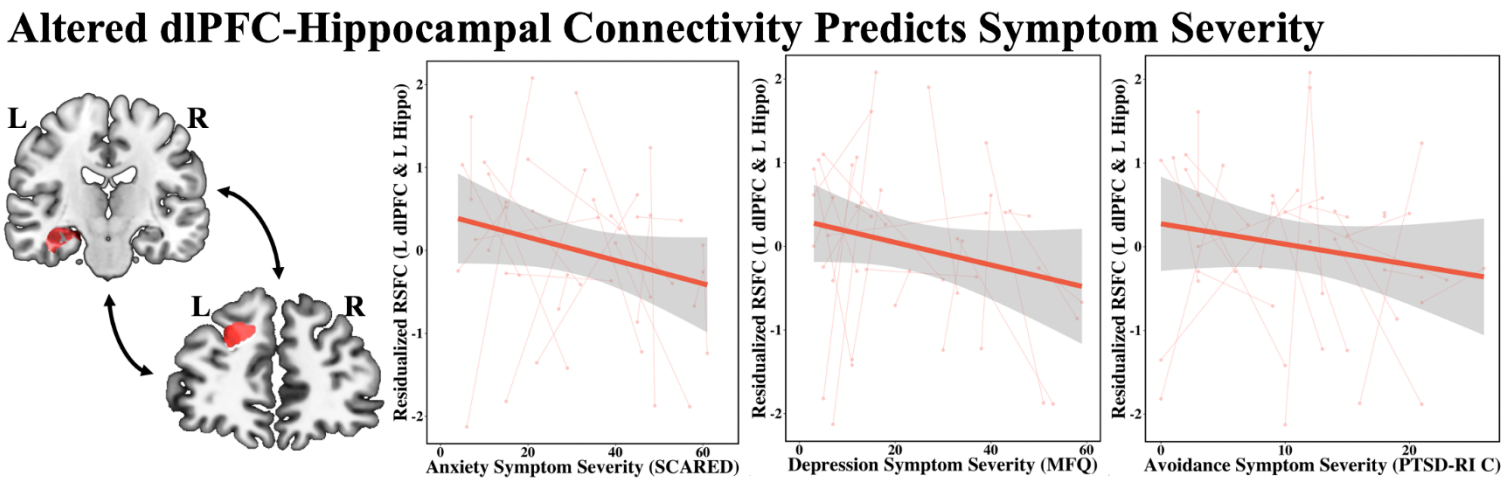

Figure S3: ***Longitudinal intrinsic connectivity abnormalities correlate with symptom severity in youth with PTSD (n=22).*** Scatterplots show extracted cluster data from trending group x time interaction between the dlPFC and anterior hippocampus in relation to anxiety, depression, and avoidance symptom severity as measured by SCARED, MFQ, and PTSD-RI Subscale C, respectively.

Abbreviations: dlPFC, dorsolateral prefrontal cortex; RSFC, resting state functional connectivity; PTSD, posttraumatic stress disorder; SCARED, Screen for Child Anxiety Related Disorders; MFQ, Mood and Feelings Questionnaire; PTSD-RI, UCLA PTSD Reaction Index.

Supplemental References

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2. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science. 2003 Aug 8;301(5634):805–9.

3. Powell TR, Murphy T, de Jong S, Lee SH, Tansey KE, Hodgson K, et al. The genome-wide expression effects of escitalopram and its relationship to neurogenesis, hippocampal volume, and antidepressant response. Am J Med Genet Part B Neuropsychiatr Genet Off Publ Int Soc Psychiatr Genet. 2017 Jun;174(4):427–34.

4. Amunts K, Kedo O, Kindler M, Pieperhoff P, Mohlberg H, Shah NJ, et al. Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. Anat Embryol (Berl). 2005 Dec;210(5–6):343–52.