Reduced Ventral Tegmental Area-Hippocampal Connectivity in Children and Adolescents Exposed to Early Threat

Supplemental Information

Imaging Data Acquisition

MRI data were acquired on a 3.0 T Siemens MAGNETOM Verio scanner (MRI Research Facility, Wayne State University School of Medicine). Six minutes of resting-state data were analyzed, during which participants were instructed to lay awake with their eyes closed. Foam padding was used to minimize motion during scanning. Data were combined across two fMRI sequences to achieve a larger sample size, however groups did not differ on sequence used, $c^2(1) = 0.341$, $p = 0.386$. In addition, sequence parameters (e.g., repetition time [TR]) were accounted for when computing individual functional connectivity values, and sequence was included as a nuisance variable in group-level analyses. Of note, sequence did not have an effect on observed functional connectivity results. Eighty-four percent of the sample (n = 72) underwent the following fMRI sequence: 180 volumes, TR = 2000 ms; echo time [TE] = 25 ms; flip angle = 90°; voxel size = 3.44 x 3.44 x 4 mm; matrix = 220 x 220 and 29 slices. The remaining participants (n = 6 trauma, n = 8 comparison) underwent the following fMRI sequence: 240 volumes, TR = 1500 ms; TE = 31 ms; flip angle = 83°; voxel size = 2.9 x 2.9 x 2.9 mm; matrix = 186 x 186 and 51 slices. Additionally, a high-resolution T1-weighted image was obtained for anatomical reference within the same imaging session.

Image Preprocessing

Image preprocessing was performed using SPM8 (Statistical Parametric Mapping; http:/www.fil.ion.ucl.ac.uk/spm/). Images were slice-time corrected, realigned, spatially

normalized to the Montreal Neurological Institute (MNI) template, and smoothed using a 6-mm Gaussian kernel.

Mitigation of Potential Motion-Related Artifact

In order to decrease the effects of motion in the functional connectivity analyses, we implemented a multi-step motion correction process: 1) functional image volumes were realigned to the mean image and 2) band-bass filtered to 0.008-0.09 Hz. 3) Realignment parameters and 4) signals from white matter and cerebral spinal fluid (aCompCor; 1) were removed using covariate regression analysis before functional connectivity was computed. 5) Analyses were repeated with the addition of motion despiking, in order to further mitigate the impact of potential outlier timepoints. We chose to remove the influence of outlying frames via despiking rather than censoring timepoints (i.e., "scrubbing") to retain an equal number of timepoints for each participant. The despiking option in CONN toolbox applies a continuous "squashing" function to the fMRI timeseries (hyperbolic tangent), applying an approximately linear squashing function to timepoints that lie within ± 3 SD around the mean and an increasingly harsher compression for timepoints beyond those limits (https://www.nitrc.org/projects/conn/). Results were consistent with those reported below (without despiking applied). 6) Mean and maximum framewise displacement (FD) were calculated as measures of movement across the scan. Importantly, mean and maximum FD did not differ between groups (see Table 1). Movement was relatively low across the sample (< 0.5 mm FD; see Table 1). 7) Group-level analyses were also repeated with mean and maximum FD entered as nuisance covariates. Results were consistent with those reported here.

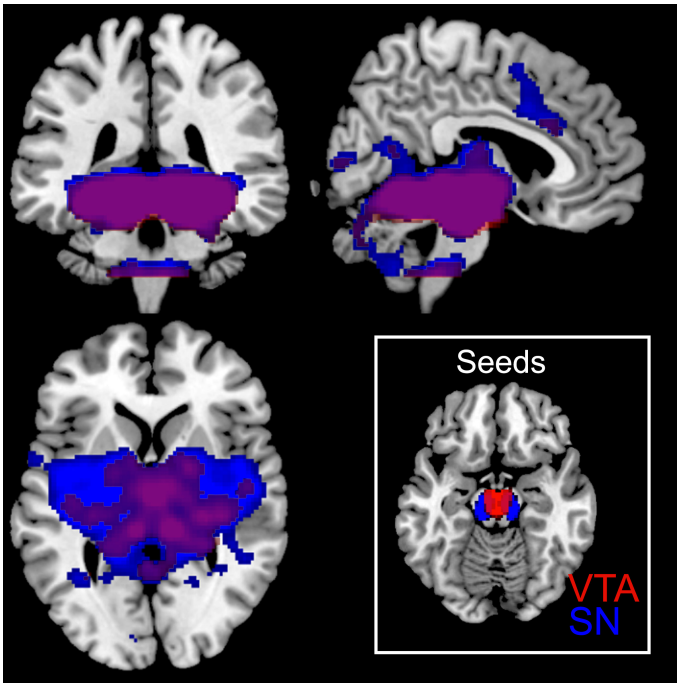

Figure S1. Functional connectivity of ventral tegmental area (VTA; red) and substantia nigra (SN; blue) midbrain regions across the sample. Overlap is shown in purple. Results of one-sample t-tests are shown here at $p < 0.005$, 10 voxel threshold for display purposes. See Table S1 for summary of results surviving whole brain correction.

Table S1. VTA and SN functional connectivity across the sample.

				MNI coordinates		
Brain region	**BA**	***Z***	**# voxels**	***x***	***y***	***z***
VTA: One-sample t-test						
Temporal lobe, cerebellum, thalamus, medial temporal lobe, insula, precuneus, occipital lobe, subgenual cingulate cortex	10/19/40/22/13/45/9/21/6/8/47/42/44/46/32	Inf	214580	-2	-14	-14
Cerebellum, brainstem	n/a	5.94	422	4	-34	-50
SN: One-sample t-test						
Temporal lobe, cerebellum, parahippocampal gyrus, insula, inferior frontal gyrus, fusiform, basal ganglia, medial temporal lobe, occipital lobe	13/22/47/21/20/38/30/19/36/37/34/35/28	Inf	282020	-14	-20	-12
Cerebellum, brainstem	n/a	6.65	927	4	-34	-50
Middle cingulate gyrus, supplemental motor area	32/24/6	4.25	264	10	12	34
Calcarine gyrus	17	3.84	145	6	-84	8
VTA > SN: Paired-sample t-test						
Brainstem, hippocampus, parahippocampal gyrus	34	Inf	867	-2	-12	-20
Precuneus, superior, middle, and inferior frontal gyri, dorsal anterior cingulate, inferior parietal lobe, basal ganglia	40/7/8/31/10/9/23/32/39	6.75	103980	-2	14	6
Cerebellum	n/a	4.93	653	50	-52	-44
Middle and superior frontal gyri	8/6/9	4.71	894	34	26	50
Cerebellum, midbrain	n/a	4.63	128	6	-40	-18
Hippocampus, middle and inferior temporal gyri	20/21	4.38	106	42	-14	-18
Superior frontal gyrus, supplemental motor area	6/8	4.36	274	-14	20	62
Inferior temporal gyrus	37/20	4.35	139	-62	-52	-14
Cerebellum	n/a	4.3	228	-32	-66	-38
Orbitofrontal cortex, gyrus rectus	11	4.29	361	-12	46	-28
Middle and superior frontal gyri	8/6/9	4.15	277	-32	28	54
Orbitofrontal cortex, gyrus rectus	11/47	4.15	108	14	44	-28
Subgenual cortex	11	4.07	95	2	28	-32
Superior and middle frontal gyri	10	3.8	145	24	56	22
SN > VTA: Paired-sample t-test						
Brainstem, temporal lobe, parietal lobe, insula, basal ganglia, thalamus	13/22/6/40/21/47/39/41/42/43/30/19	Inf	311360	-14	-20	-8
Supplemental motor area, paracentral lobule	6/24/32	5.23	1666	-2	-12	52
Subgenual cingulate cortex, orbitofrontal cortex, ventromedial prefrontal cortex	11/25/32	4.86	377	0	28	-14
Brainstem	n/a	4.32	120	-8	-28	-42
Cerebellum, brainstem	n/a	4.12	193	4	-60	-34

All coordinates survive whole brain correction. One-sample t-tests use bivariate correlation values, and denote positive connectivity of the seed region across the brain. BA, Brodmann area; MNI, Montreal Neurological Institute.

Table S2. Whole brain effects of age on functional connectivity of VTA and SN.

				MNI coordinates		
Brain region	**BA**	***Z***	**# voxels**	***x***	***y***	***z***
VTA: Positive age effects						
No clusters survive whole brain correction						
VTA: Negative age effects						
Hippocampus, parahippocampal gyrus	20	4.66	451	38	-32	-12
Inferior parietal lobe	40/7	4.61	150	-54	-50	52
Parahippocampal gyrus, hippocampus, fusiform	35	4.2	338	-34	-24	-18
SN: Positive age effects						
No clusters survive whole brain correction						
SN: Negative age effects						
Inferior parietal lobe	40	5.15	192	-52	-50	50
Hippocampus, parahippocampal gyrus, cerebellum	35	4.63	344	-30	-30	-12
Parahippocampal gyrus, hippocampus, thalamus, globus pallidus	36	4.48	358	34	-28	-14
Midbrain, cerebellum	n/a	4.07	177	-2	-26	-14

All coordinates survive whole brain correction. BA, Brodmann area; MNI, Montreal Neurological Institute.

Table S3. Whole brain effects of clinical measures on functional connectivity of VTA and SN.

				MNI coordinates		
Brain region	**BA**	***Z***	**# voxels**	***x***	***y***	***z***
Anxiety symptoms						
VTA: Increased anxiety symptoms increased connectivity						
Middle cingulate cortex	24	4.67	96	4	4	22
VTA: Increased anxiety symptoms decreased connectivity						
Dorsomedial prefrontal cortex	8	4.32	318	18	32	36
SN: Increased anxiety symptoms increased connectivity						
No clusters survive whole brain correction						
SN: Increased anxiety symptoms decreased connectivity						
Dorsomedial prefrontal cortex	8	4.38	122	20	22	42
Reward sensitivity (RS)						
VTA: Increased RS increased connectivity						
No clusters survive whole brain correction						
VTA: Increased RS decreased connectivity						
No clusters survive whole brain correction						
SN: Increased RS increased connectivity						
Middle occipital gyrus	18,19	4.16	141	-26	-94	10
Middle occipital gyrus	18,19	4.24	94	26	-88	0
SN: Increased RS decreased connectivity						
No clusters survive whole brain correction						
Depressive symptoms						
VTA: Increased depressive symptoms increased connectivity						
No clusters survive whole brain correction						
VTA: Increased depressive symptoms decreased connectivity						
No clusters survive whole brain correction						
SN: Increased depressive symptoms increased connectivity						
No clusters survive whole brain correction.						
SN: Increased depressive symptoms decreased connectivity						
No clusters survive whole brain correction						

All coordinates survive whole brain correction. BA, Brodmann area; MNI, Montreal Neurological Institute.

Supplemental Reference

1. Muschelli J, Nebel MB, Caffo BS, Barber AD, Pekar JJ, Mostofsky SH (2014): Reduction of motion-related artifacts in resting state fMRI using aCompCor. *NeuroImage*. 96:22-35.