**Supplementary material**

1. **Measures**
2. **Additional analysis of structural data**
3. **Supplementary tables**
4. **Supplementary figures**

**1. Measures**

Before MR scanning, each participant was assessed with a set of neuropsychological tests, which included the Hamilton Depression (HAMD) (Hamilton, 1960) and Hamilton Anxiety (HAMA) (Hamilton, 1959) rating scales, and the Mini-Mental State Examination (MMSE) (Folstein *et al.*, 1983). The bereaved parents also had their social support level assessed using the Chinese Social Support Rating Scale (SSRS) (Cheng *et al.*, 2008); individual coping ability was also evaluated with the Simple Coping Style Questionnaire (SCSQ) (Jiang *et al.*, 2017). In detail, the SSRS contains three subscales of social support: subjective support (refers to perceived interpersonal network that an individual can count on, 4 items with scores ranging from 8–32); objective support (actual support an individual received, 3 items with scores ranging from 1–22); and the utility of support (the pattern of behavior that an individual use when seeking social support, 3 items with scores ranging from 3–12). Higher scores for the SSRS indicate stronger social support, and the total support score (ranging from 12–66) comes from the sum of all the sub-items. The SCSQ contains assessments of both active and negative coping, containing 12 and 8 items, respectively. The scale of each SCSQ item uses 4-level Likert score standards, in which ‘3’ stands for regular use, while ‘0’ stands for no use. Then, the scores for active and negative coping are calculated independently, and a higher score indicates the inclination to adopt the corresponding coping style, while the coping tendency score is defined as the active minus negative coping score.

**2. Additional analysis of structural data**

The relationship between hippocampal volume and PTSD is currently controversial in the literature (Logue *et al.*, 2018, McNerney *et al.*, 2018). To evaluate the possible confounding effect of hippocampal atrophy on the functional results, we further conducted voxel-based morphometry (VBM) analysis to examine possible hippocampal structural deficits in a similar way to that used in a prior study of PTSD (Chen and Etkin, 2013), within which no differences in hippocampal subregional volumes were detected. VBM was performed using the CAT12 Toolbox (http://dbm.neuro.uni-jena.de/cat12/). We used the default settings detailed in the manual for CAT12 toolbox (http://dbm.neuro.uni-jena.de/cat12/CAT12-Manual.pdf) except for applying the affine regularisation using the International Consortium for Brain Mapping template for East Asian brains. The individual T1-weighted images underwent bias-correction, tissue classification, and were transformed into standard MNI space. The segmented, modulated gray matter (GM) images were smoothed with an FWHM of 8 mm. The volume of each hippocampal subregion was extracted for statistical analysis.

We performed ANOVA to assess possible volume differences in hippocampal subregions among three groups. A voxel-wise comparison was further conducted across the three groups to determine any differences in regional GM volumes across the whole brain.VBM analysis indicated that there were no detectable gray matter volume differences in any hippocampal subregions among the three groups (ANOVA: *f* = 0.38, *p* =0.70 for left hippocampal CA1; *f* = 0.31, *p* = 0.97 for right CA1; *f* = 0.65, *p* = 0.94 for left CA2; *f* = 0.13, *p* = 0.88 for right CA2; *f* = 0.41, *p* = 0.66 for left CA3; *f* = 0.03, *p* = 0.99 for right CA3; *f* = 0.51, *p* = 0.60 for left DG; *f* = 0.84, *p* = 0.92 for right DG). Also, we found no gray matter volume differences in a voxel-wise ANOVA across the whole brain (total intracranial volume was included as a covariate). Furthermore, the results of CA1 and DG functional connectivity among PTSD, non-PTSD and healthy control group remained significant even when controlling for participant’ hippocampal subregional GM volume (data not shown). Thus, hippocampal functional connectivity results in the current study were not due to volumetric differences, at least not affected by the VBM measurements.

VBM = voxel-based morphometry; ANOVA = analysis of variance; CA = *cornu ammonis*; DG = *dentate gyrus*; PTSD = post-traumatic stress disorder.

**3. Supplementary tables**

**Table S1.** Primer sequences for the *NR3C1* gene (start and end sites were named with their relative distance to the TSS)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| PCR  size(bp) | Start site | End  site | Primer |  |
| 285 | -1005 | -836 | forward | GYGAATTTTTGTTAAGATGGTGGT |
|  |  |  | reverse | CCACAACCACTCTCTCACCTC |
| 1225 | +215 | +393 | forward | ATTYGGGAGTTYGTTTTGTTTTT |
|  |  |  | reverse | TACAACCCCRTAACCCCTTTC | |
|  | +889 | +1092 | forward | TTTTATTTTGYGAGTTYGTGTTTGTG |
|  |  |  | reverse | AATCTCCCATTACCCAACTAACAAA |
| 796 | +1315 | +1471 | forward | GGAGGGAGAGGAAGAGGTTAG |
|  |  |  | reverse | AAATACCRCTAAAACCRAAAACAACTC |

TSS = transcriptional start site; PCR = Polymerase chain reaction.

**Table S2.** Methylated CpG sites of the *NR3C1* gene identified in this study.

| Position | Genomic location\* | Relative to TSS, bp |
| --- | --- | --- |
| 1 | Chr5:142785026 | -981 |
| 2 | Chr5:142785024 | -979 |
| 3 | Chr5:142785018 | -973 |
| 4 | Chr5:142785010 | -965 |
| 5 | Chr5:142784986 | -941 |
| 6 | Chr5:142784983 | -938 |
| 7 | Chr5:142784979 | -934 |
| 8 | Chr5:142784972 | -927 |
| 9 | Chr5:142784967 | -922 |
| 10 | Chr5:142784965 | -920 |
| 11 | Chr5:142784953 | -908 |
| 12 | Chr5:142784924 | -879 |
| 13 | Chr5:142784921 | -876 |
| 14 | Chr5:142784909 | -864 |
| 15 | Chr5:142784907 | -862 |
| 16 | Chr5:142784904 | -859 |
| 17 | Chr5:142783678 | 367 |
| 18 | Chr5:142783685 | 360 |
| 19 | Chr5:142783688 | 357 |
| 20 | Chr5:142783702 | 343 |
| 21 | Chr5:142783712 | 333 |
| 22 | Chr5:142783716 | 329 |
| 23 | Chr5:142783730 | 315 |
| 24 | Chr5:142783735 | 310 |
| 25 | Chr5:142783742 | 303 |
| 26 | Chr5:142783744 | 301 |
| 27 | Chr5:142783755 | 290 |
| 28 | Chr5:142783766 | 279 |
| 29 | Chr5:142783768 | 277 |
| 30 | Chr5:142783771 | 274 |
| 31 | Chr5:142783774 | 271 |
| 32 | Chr5:142783777 | 268 |
| 33 | Chr5:142783780 | 265 |
| 34 | Chr5:142783785 | 260 |
| 35 | Chr5:142783792 | 253 |
| 36 | Chr5:142783809 | 236 |
| 37 | Chr5:142783129 | 916 |
| 38 | Chr5:142783121 | 924 |
| 39 | Chr5:142783113 | 932 |
| 40 | Chr5:142783105 | 940 |
| 41 | Chr5:142783102 | 943 |
| 42 | Chr5:142783096 | 949 |
| 43 | Chr5:142783073 | 972 |
| 44 | Chr5:142783070 | 975 |
| 45 | Chr5:142783064 | 981 |
| 46 | Chr5:142783059 | 986 |
| 47 | Chr5:142783055 | 990 |
| 48 | Chr5:142783040 | 1005 |
| 49 | Chr5:142783035 | 1010 |
| 50 | Chr5:142783028 | 1017 |
| 51 | Chr5:142783026 | 1019 |
| 52 | Chr5:142783024 | 1021 |
| 53 | Chr5:142783020 | 1025 |
| 54 | Chr5:142783012 | 1033 |
| 55 | Chr5:142783007 | 1038 |
| 56 | Chr5:142783005 | 1040 |
| 57 | Chr5:142782998 | 1047 |
| 58 | Chr5:142782995 | 1050 |
| 59 | Chr5:142782993 | 1052 |
| 60 | Chr5:142782988 | 1057 |
| 61 | Chr5:142782595 | 1450 |
| 62 | Chr5:142782605 | 1440 |
| 63 | Chr5:142782607 | 1438 |
| 64 | Chr5:142782609 | 1436 |
| 65 | Chr5:142782620 | 1425 |
| 66 | Chr5:142782626 | 1419 |
| 67 | Chr5:142782629 | 1416 |
| 68 | Chr5:142782633 | 1412 |
| 69 | Chr5:142782664 | 1381 |
| 70 | Chr5:142782691 | 1354 |
| 71 | Chr5:142782693 | 1352 |
| 72 | Chr5:142782696 | 1349 |
| 73 | Chr5:142782703 | 1342 |

\*The chromosomal location of each CpG site according to assembly GRCh37.p13.

TSS = transcriptional start site; Chr = chromosome.

**Table S3:** Positive FC of left hippocampal CA1 (*p* < 0.05, corrected)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Brain regions | BA | MNI coordinates (mm) | Voxel number | Statistic values |
| (x, y, z) |
| **ANOVA** |  |  |  | *f* value |
| PCC | 31 | -3,-51,18 | 96 | 5.44 |
| **PTSD versus HC** |  |  |  | *t* value# |
| PCC | 31 | -6,-57,30 | 94 | -2.87 |
| **non-PTSD versus HC** |  |  |  | *t* value# |
| PCC | 31 | 0,-51,18 | 94 | -3.09 |

FC = functional connectivity; CA = *cornu ammonis*; ANOVA = analysis of variance; BA= Brodmann area; MNI = Montreal Neurologic Institute; PTSD = post-traumatic stress disorder; HC = healthy controls; PCC = posterior cingulate cortex. # Negative sign represents decrease.

**Table S4:** Positive FC of left hippocampal DG (*p* < 0.05, corrected)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Brain regions | BA | MNI coordinates (mm) | Voxel number | Statistic values |
| (x, y, z) |
| **ANOVA** |  |  |  | *f* value |
| PCC | 31 | -3,-48,18 | 64 | 5.98 |
| **PTSD versus HC** |  |  |  | *t* value# |
| PCC | 31 | 3,-18,15 | 57 | -2.69 |
| **non-PTSD versus HC** |  |  |  | *t* value# |
| PCC | 31 | -3,-48, 18 | 49 | -3.31 |

FC = functional connectivity; DG = *dentate gyrus*; ANOVA = analysis of variance; BA = Brodmann area; MNI = Montreal Neurologic Institute; PTSD = post-traumatic stress disorder; HC = healthy controls; PCC = posterior cingulate cortex. # Negative sign represents decrease.

**Table S5:** Negative FC of right hippocampal CA1 (*p* < 0.05, corrected)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Brain regions | BA | MNI coordinates (mm) | Voxel number | Statistic values |
| (x, y, z) |
| **ANOVA** |  |  |  | *f* value |
| MFC | 32/9 | -3,33,33 | 67 | 8.31 |
| MFG/IFG\_R | 10/46 | 39,39,18 | 134 | 8.17 |
| **PTSD versus HC** |  |  |  | *t* value# |
| MFC | 32/9 | -3,33,33 | 67 | -2.76 |
| MFG/IFG\_R | 10/46 | 39,39,18 | 132 | -3.83 |
| **non-PTSD versus HC** |  |  |  | *t* value# |
| MFC | 32/9 | -3,33,33 | 67 | -3.91 |
| MFG/IFG\_R | 10/46 | 39,39,18 | 76 | -2.85 |
| **PTSD versus non-PTSD** |  |  |  | *t* value# |
| MFG/IFG\_R | 10/46 | 45,45,15 | 58 | -2.82 |

FC = functional connectivity; CA = *cornu ammonis*; ANOVA = analysis of variance; BA = Brodmann area; MNI = Montreal Neurologic Institute; PTSD = post-traumatic stress disorder; HC = healthy controls; MFC = medial frontal cortex; MFG/IFG = middle frontal gyrus/inferior frontal gyrus. # Negative sign represents decrease.

**Table S6:** Negative FC of right hippocampal DG (*p* < 0.05, corrected)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Brain regions | BA | MNI coordinates (mm) | Voxel number | Statistic values |
| (x, y, z) |
| **ANOVA** |  |  |  | *f* value |
| SFG\_R | 8 | 0,30,51 | 64 | 5.81 |
| MFC | 9/32 | -3,30,33 | 77 | 6.28 |
| MFG\_L | 10 | -39,51,0 | 65 | 5.50 |
| MFG\_R | 46/10 | 39,39,18 | 117 | 7.19 |
| **PTSD versus HC** |  |  |  | *t* value# |
| SFG\_R | 8 | 0,30,51 | 51 | -3.34 |
| MFC | 9 | 12,27,39 | 68 | -3.24 |
| MFG\_L | 10 | -39,51,3 | 65 | -3.09 |
| MFG\_R | 46/10 | 39,39,18 | 89 | -3.48 |
| **non-PTSD versus HC** |  |  |  | *t* value# |
| SFG\_R | 8 | 6,18,57 | 26 | -2.56 |
| MFC | 9 | -3,30,33 | 67 | -3.47 |
| MFG\_R | 46/10 | 36,36,21 | 101 | -3.05 |
| **PTSD versus non-PTSD** |  |  |  | *t* value# |
| SFG\_R | 8 | 3,24,54 | 26 | -2.28 |
| MFG\_L | 10 | -39,51,-3 | 51 | -3.15 |

FC = functional connectivity; DG = dentate gyrus; ANOVA = analysis of variance; BA= Brodmann area; MNI = Montreal Neurologic Institute; PTSD = post-traumatic stress disorder; HC=healthy controls; SFG = superior frontal gyrus; MFC = medial frontal cortex; MFG = middle frontal gyrus. # Negative sign represents decrease.

**4. Supplementary figures**

**Figure S1.** Illustration of the seed regions of interest (ROIs) of the hippocampus.



Bilateral CA1, CA2, CA3, and DG are used in the present functional connectivity analyses.

CA = *cornu ammonis*; DG = *dentate gyrus*.

**Figure S2.** CpG regions sequenced in the *NR3C1* gene.

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Blue lines with arrows indicate selected CpG regions conducted in the current study. The range of each region is indicated by its relative distance (in bp) to the transcriptional start site (TSS).

**Figure S3.** The differences in mean and individual methylation levels of the *NR3C1* gene among PTSD, non-PTSD, and healthy controls.

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When compared with never-traumatized healthy controls, both PTSD and non-PTSD parents show lower mean percentage of methylation of the *NR3C1* gene. There is no significant difference of mean methylation levels between these two trauma-exposed groups. Following individual analyses at each CpG site show that all 7 CpG sites have differences of methylation levels among the three groups. For most of these CpG sites, lower methylation levels are found in PTSD (sites 1, 17, 30, 34 and 51) and also in non-PTSD groups (sites 1, 12, 17, 34 and 51), compared to the HC group. Only two CpG sites (sites 1 and 6) display lower methylation levels in PTSD group than non-PTSD group.

PTSD = post-traumatic stress disorder.

**Figure S4.** The correlation results.

In non-PTSD adults, significant positive correlation is discovered between CAPS-B re-experiencing subscale scores and the FC of the right DG–SFG and MFC.



FC = functional connectivity; PTSD = post-traumatic stress disorder; CAPS = clinician-administered PTSD scale; Hip = hippocampal; DG = *dentate gyrus*; SFG = superior frontal gyrus; MFC = medial frontal cortex.

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