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Hippocampal subfields alterations in adolescents with post-traumatic stress disorder

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Short title: Hippocampal subfields in adolescent PTSD

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Abstract

Re-experiencing symptoms in adolescent Post-Traumatic Stress Disorder (PTSD) are characterized by the apparition of vivid intrusive images of the traumatic event. The emergence of these intrusions is thought to be related to a deficient context processing and could then be related to hippocampal alterations. The hippocampus is a complex structure which can be divided into several subfields, namely the Cornu Ammonis (CA1, CA2 and CA3), the subiculum and the dentate gyrus (DG). As each subfield present different histological characteristics and functions, it appears more relevant to consider hippocampal subfields, instead of only assessing the whole hippocampus, to understand the neurobiology of PTSD. Hence, this study presents the first investigation of structural alterations within hippocampal subfields and their links to re-experiencing symptoms in adolescent PTSD. Hippocampal subfields were manually delineated on high resolution MRI images in 15 adolescents (13-18 years old) with PTSD and 24 age-matched healthy controls. The volume of the region CA2-3/DG region was significantly smaller in the PTSD group compared to controls in both hemispheres. No other significant difference was found for other subfields. Moreover, the volume of the left CA2-3/DG was negatively correlated with the intrusion score (as measured by the Impact of Events Scale-Revised) in the PTSD group. To conclude, an alteration in the hippocampal subregion CA2-3/DG, known to resolve interferences between new and similar stored memories, could participate in the apparition of intrusive trauma memories in adolescents with PTSD.

Keywords: hippocampal subfields; adolescents; MRI; post-traumatic stress disorder

Introduction

Individuals who lived a traumatic experience are at risk of developing a post-traumatic stress disorder (PTSD). This psychiatric disorder is associated with re-experiencing symptoms, which are characterized by the intrusions of vivid and involuntary memories of the traumatic event. PTSD symptoms also encompass avoidance of trauma-reminders, excessive physiological arousal and negative alteration in cognition and mood [American Psychiatric Association, 2013]. A deficient context processing could be at the core of PTSD pathophysiology [Liberzon and Abelson, 2016]. Processing contextual information is crucial to disambiguate cues associated with safety and threat and, consequently, adapt our behavior to the environment [Liberzon and Abelson, 2016; Maren et al., 2013]. Hence, a deficient context processing could participate in the emergence of recurrent intrusive memories and the overgeneralization of fear responses in safe environments [Liberzon and Abelson, 2016]. These intrusions could be related to an alteration of the hippocampal structure, known to be involved in contextual memory [Maren et al., 2013]. Indeed, several neuroimaging studies reported smaller hippocampal volumes in adults suffering from PTSD compared to people who lived a traumatic experience without PTSD and compared to healthy controls [Logue et al., 2018; Pitman et al., 2012].

The hippocampus is a complex structure which can be divided into several subfields, namely the Cornu Ammonis (CA1, CA2 and CA3), the subiculum and the dentate gyrus [Duvernoy, 2005]. These hippocampal subfields have distinct histological characteristics and present, thereby, differential vulnerability to pathological conditions such as Alzheimer's disease, schizophrenia and major depression [Small et al., 2011] and to other factors like stress [Gould, 2007]. Furthermore, hippocampal subfields are involved in different memory processes. It has been shown that CA1 plays a role in pattern completion (ability to recall a memory based on a partial cue) [Carr et al., 2010]. The dentate gyrus (DG) has a function in

pattern separation (ability to diminish the similarity between two resembling memories) and CA3 is thought to be involved in both processes. Hence, investigating hippocampal subregions, instead of the hippocampus as a single entity, appears more relevant to understand the neurobiology of PTSD and the mechanisms underlying the re-experiencing symptoms.

Alterations within hippocampal subregions can be examined in volumetric studies using high resolution Magnetic Resonance Imaging (MRI). Wang *et al.* [Wang et al., 2010] manually segmented a part of the hippocampal body and found that only the CA3/DG volume was significantly smaller in adults with PTSD compared to veterans without PTSD. Although they did not investigate subfield volumes of the entire hippocampus, they suggested that PTSD was not related to a global atrophy of the hippocampus but to alterations of these specific subfields [Wang et al., 2010]. These results are in line with theoretical models which propose that re-experiencing symptoms could be related to a pattern separation dysfunction resulting from a reduced neurogenesis in the dentate gyrus [Besnard and Sahay, 2016; Liberzon and Abelson, 2016].

To our knowledge, there is no existing investigation of hippocampal subfield alterations in adolescent PTSD. This could result from the consistent finding that the hippocampus is not impaired in children and adolescents with PTSD. Unlike work in adults, most pediatric neuroimaging studies do not report any significant difference in hippocampal volume in children and adolescents with PTSD compared to controls [Morey et al., 2016; Woon and Hedges, 2008] (but see [Mutluer et al., 2017]). Nonetheless, these studies may have failed to detect any alteration because of the use of low resolution structural MRI images or processing techniques not adapted to pediatric populations [Keding and Herringa, 2015]. For instance, the automatic segmentation method freesurfer is known to over-estimate hippocampal volumes in children [Schoemaker et al., 2016]. In addition, even if there are no global changes detected on the hippocampus, it does not mean that there are no local effects

on hippocampal subfields. For example, one study [Gogtay et al., 2006] showed that the volume of the whole hippocampus in four years old was similar to young adults, but recent volumetric studies revealed differential ongoing maturation of the hippocampal subfields during childhood and adolescence [Daugherty et al., 2017; Lee et al., 2014]. Hence, even if there is no alteration of the volume of the whole hippocampus, PTSD symptomatology in adolescents may be associated with alterations of specific hippocampal subfields.

This present study aims to examine the volume of the hippocampus as a whole and hippocampal subfields in adolescent PTSD. The hippocampus and its subfields were manually segmented on high resolution 3T MRI images and, then, their volumes were extracted. We predicted smaller volumes of the CA3 and DG subfields in adolescents with PTSD compared to controls. We also hypothesized that these alterations would be related to re-experiencing symptoms.

Methods and materials

Participants

Fifteen adolescents with PTSD, aged 13 to 18 years (13 females), were recruited through the departments of child and adolescent psychiatry of three French University Hospitals (Caen, Rennes, Rouen). PTSD adolescents received no psychotropic medication during the previous week and were free from other mental disorders including major depression. Twenty-five typically developing adolescents (12 females) with no history of trauma were recruited by prospecting in several junior high schools of the region (Normandy, France). One subject was excluded due to severe motion artifacts on the MRI data.

Hence, 15 PTSD patients and 24 controls were included in the analyses (see Table 1). Some participants for this study took part in a larger project investigating self-reference processing [Dégeilh et al., 2017]. All were right-handed and French native speakers. None of

them reported any prior or neurological or learning disabilities, head trauma, and MRI contraindications. The study was approved by the local Ethics Committee (CPP Nord Ouest III). All adolescents and their parents signed informed consent after a comprehensive description of the study.

Assessment

A board-certified child and adolescent psychiatrist interviewed and screened all participants. Psychiatric diagnoses were assessed using the Structured Clinical Interview-Clinician Version (SCID-CV) [First et al., 1996; Lobbestael et al., 2011]. PTSD severity was additionally examined with the French version of the Impact of the Event Scale Revised (IES-R) [Brunet et al., 2003; Weiss and Marma, 1997]. This scale assessed intrusions, hyperarousal and avoidance symptoms. Major depression was categorically screened using the SCID-CV [First et al., 1996; Lobbestael et al., 2011] and dimensionally measured with the French version of the Children Depression Inventory (CDI) [Dugas and Bouvard, 1996; Kovacs, 1981].

Neuroimaging data acquisition

All participants were scanned with a 3T Philips MRI scanner at the Cyceron Center (Caen, France). T1-weighted anatomical volumes were acquired using a three-dimensional fast-field echo sequence (3D-T1-FFE sagittal; Repetition Time (RT) = 20 ms; Echo Time (ET) = 4.6 ms; flip angle = 10°; 180 slices; slice thickness = 1 mm; field of view = 256 × 256 mm², in-plane resolution = 1 × 1 mm²; acquisition time = 9min 41s). In order to segment the hippocampal subfields, a high resolution proton density weighted sequence was acquired perpendicularly to the long axis of the hippocampus (Repetition Time (RT) = 3,500 ms; Echo Time (ET) = 19 ms; flip angle = 90°; 13 slices; slice thickness = 2 mm; interslice gap = 2 mm; in-plane resolution = 0.375 × 0.375 mm²; acquisition time = 7min 38s).

Manual segmentation of hippocampal subfields

Hippocampal subfields were manually demarcated following the procedure developed in our laboratory [de Flores et al., 2015; La Joie et al., 2010]. From the most anterior part of the hippocampus to the apparition of colliculi, the hippocampus was segmented into three areas: (1) Subiculum; (2) CA1; and (3) a region combining CA2, CA3 and the DG (“CA2-3/DG”). Because it is particularly difficult to differentiate the subfields in the tail of the hippocampus, the most posterior part of the hippocampus (posterior to the colliculi) was segmented into one single region called “tail”. The volume of the whole hippocampus corresponded to the sum of the four segmented subregions (subiculum, CA1, CA2-3/DG, tail). Manual delineations were all performed by the same rater (CP), blind to any information concerning the participants. Then, a second rater (CA), also blind to the identity of the participants, visually checked and validated all the delineations. Before segmenting the data, CP was trained during several months by two expert raters (RDF and CA). The formation was validated by a high inter-rater reliability between CP and CA on an independent sample of 20 healthy subjects (Intraclass Correlation Coefficients: $ICC(2;1) = 0.94, 0.91$ and 0.91 for CA1, Subiculum and CA2-3/DG respectively).

Statistical analyses

Statistical analyses were performed using Statistica 13 (Statsoft, Tulsa, USA). First, raw volumes were normalized to the total intracranial volume (TIV) in order to correct for head-size differences [de Flores et al., 2015; La Joie et al., 2010]. TIV values were extracted from the T1-weighted images with the Computational Anatomy Toolbox (CAT12). To evaluate group differences, we performed general linear models (GLMs) with the TIV-adjusted volumes as dependent variables, diagnosis as independent variable and age, sex and the depression score (CDI) as nuisance covariates. We included the depression score as nuisance

covariate because depression were previously reported as related to structural changes in hippocampal subfields [Huang et al., 2013].

Then, when the hippocampal subfields volumes were significantly different between groups, we conducted partial correlations (nuisance covariates: age, sex, depression score) between the TIV-adjusted volumes and the three scores (intrusion, hyperarousal and avoidance) of the IES-R scale assessing for symptom severity. FDR correction was applied to adjust for multiple comparisons.

Results

Participant characteristics

Participant characteristics are displayed in Table 1. There were no significant differences between groups for age and IQ. PTSD patients showed significantly higher levels of symptom severity on the three scores of the IES-R scale (intrusion: $t = 4.76$, $p < 0.001$; hyperarousal: $t = 6.26$, $p < 0.001$; and avoidance: $t = 4.07$, $p < 0.001$) compared to controls. They also had a higher score on the depression scale (CDI score: $t = 4.57$, $p < 0.001$) compared to controls.

PTSD effect on hippocampal subfields

Results of the TIV-adjusted volumes (mean \pm SD) of the hippocampal subfields and GLMs are summarized in Table 2. All statistical analyses were performed controlling for age, sex and depression. Concerning the whole hippocampus, the volumes of left and right hippocampi were significantly smaller in the PTSD group compared to controls. Concerning hippocampal subfields, no significant differences were found between the groups for the subiculum and CA1 volumes. However, the CA2-3/DG volumes were smaller in the PTSD group compared to controls on both (left and right) sides (Figure 1).

Correlational analyses explored the relation between the CA2-3/DG volumes and symptom severity. The left CA2-3/DG was negatively correlated with the intrusion score ($r = -0.74$, $p = 0.006$, $p_{FDR} = 0.036$) (Figure 1) but was not correlated with the hyperarousal score ($r = -0.50$, $p = 0.099$, $p_{FDR} = 0.297$), nor with the avoidance score ($r = -0.19$, $p = 0.55$, $p_{FDR} = 0.66$). The right CA2-3/DG was not correlated with any of the three IES-R scores (intrusion score: $r = -0.36$, $p = 0.26$, $p_{FDR} = 0.52$; hyperarousal score: $r = -0.14$, $p = 0.66$, $p_{FDR} = 0.66$; avoidance score: $r = 0.24$, $p = 0.46$, $p_{FDR} = 0.66$).

Of note, the significant results remain unchanged if we only consider the female subjects ($n_{PTSD} = 13$, $n_{Controls} = 12$), controlling for age and depression. The hippocampal volumes are significantly smaller in adolescents with PTSD compared to controls (left hippocampus: $F = 11.70$, $p = 0.003$; right hippocampus: $F = 18.25$, $p = 0.0003$). The CA2-3/DG regions are significantly smaller in the PTSD group compared to controls (left CA2-3/DG: $F = 9.18$, $p = 0.006$; right CA2-3/DG: $F = 13.68$, $p = 0.001$). And the left CA2-3/DG volume is negatively correlated with the intrusion score ($r = -0.74$, $p = 0.008$)

Discussion

This study presents the first investigation of alterations within hippocampal subfields and their links to re-experiencing symptoms in adolescent PTSD. Using high resolution MRI and manual segmentation, we show that the hippocampal volumes are smaller in adolescents with PTSD compared to controls and that these alterations are specific to the CA2-3/DG region. Noteworthy, these differences between groups are significant controlling for depression symptoms. In addition, we report a negative correlation between the intrusions score and the left CA2-3/DG volume in the PTSD group. Our results suggest that an alteration in the hippocampal subregion CA2-CA3/DG could participate in the emergence of re-experiencing symptoms in adolescents with PTSD.

Hippocampal volumes were smaller in adolescents with PTSD compared to controls. PTSD has been previously associated with hippocampal alterations in adults. Indeed, the ENIGMA consortium reported smaller hippocampal volumes on a large cohort (1868 subjects) in civilians and veterans with PTSD compared to controls and, a negative correlation between hippocampal volumes and PTSD severity [Logue et al., 2018]. In contrast, most of pediatric studies failed to report any hippocampal alterations in children and adolescents with PTSD compared to healthy subjects [Morey et al., 2016; Woon and Hedges, 2008] (but see [Mutluer et al., 2017]). Woon & Hedges [Woon and Hedges, 2008] hypothesized that the trauma and the development of PTSD could have a subsequent effect on hippocampal maturation and thus, the atrophy could only be detectable in adults. However, these studies could have failed to detect any alteration because of the use of low resolution structural MRI images or segmentation techniques that may not have been adapted to pediatric populations. Indeed, using high resolution MRI images and manual segmentation, we found smaller hippocampal volumes in adolescents with PTSD, in accordance with Mutluer *et al.* [Mutluer et al., 2017] who also manually segmented the whole hippocampus in adolescents with PTSD.

Furthermore, our results showed that hippocampal alterations in PTSD adolescents are specific to the CA2-3/DG region. These findings are in line with Wang *et al.* [Wang et al., 2010] who found smaller CA3/DG volumes in adults with PTSD compared to veterans. Hippocampal subfields have distinctive histological characteristics. Interestingly, neurogenesis in the dentate gyrus is thought to be present during the entire life span [Boldrini et al., 2018; Eriksson et al., 1998; Spalding et al., 2013], but this process can be negatively affected in diverse pathological conditions. Hippocampal differences described in PTSD populations could then be related to the development of comorbidities, such as major depression (see [Huang et al., 2013]). In our study, although screening excluded patients with major depression, they presented a higher score on the depression scale (CDI) compared to

controls. Depression was thus added as a nuisance covariate in all our analyses. Even controlling for depression, we found smaller volumes of the CA2-3/DG region in the PTSD group. This result suggests that hippocampal alterations are related to PTSD and are not exclusively associated with other comorbidities, like depression.

Mechanisms underlying hippocampal alterations in PTSD still remain elusive: the smaller hippocampal volumes reported in patients could result from trauma exposure or be a pre-existing condition and, thus, be a vulnerability factor for the development of PTSD [Gilbertson et al., 2002; Pitman et al., 2012]. First, hippocampal alterations could be caused by the massive amount of stress generated during the traumatic event. Indeed, the hippocampus comprises many glucocorticoid receptors and pathological stress is known to reduce dendritic branching in the Cornu Ammonis and to negatively affect neurogenesis [Gould, 2007; Schoenfeld et al., 2017]. Nonetheless, CA3/DG modifications are thought to be reversible after a certain time without stress exposure [Gould, 2007; Heine et al., 2004]. Hence, the small volumes of CA2-3/DG reported in the PTSD group could reflect a deficient process of recovery from trauma exposure.

However, one study, realized in identical twins discordant for trauma exposure, suggests that PTSD patients could already have smaller hippocampi before the trauma [Gilbertson et al., 2002; Pitman et al., 2012]. They found that the veterans with PTSD and their unexposed co-twins had similar hippocampal volumes and these two groups had smaller hippocampal volumes compared to the veterans without PTSD and the unexposed non-PTSD co-twins. Hence, having small hippocampal volumes, and especially small CA2-3/DG volumes, could be considered as a risk factor to develop PTSD. According to Kempermann's hypothesis [Kempermann, 2008], the newborn hippocampal neurons (in the dentate gyrus) constitute a “neurogenic reserve” and could be considered as an adaptive advantage to face novelty and complex situations. This neural reserve is also thought to have a compensatory

potential to maintain the hippocampal function in pathological conditions. Hence, the initial neurogenesis capacity in the dentate gyrus (before the trauma) could influence the way a person will face the traumatic event and overcome the potential consequences. In line with this hypothesis, Hill *et al.* [Hill et al., 2015] showed that mice with initial increased neurogenesis developed less anxiety-related behaviors compared to controls during a chronic stress paradigm. Further work is required to understand to which extent an enhanced neurogenesis capacity in the dentate gyrus (before the trauma) could be protective, help to overcome the alterations caused by the stress of the traumatic event and, thus, prevent the development of PTSD.

It is widely acknowledged that the hippocampus and its different subfields play a crucial role in memory processes [Horner and Doeller, 2017; Rolls, 2016]. The dentate gyrus is thought to underlie pattern separation [Berron et al., 2016] and thus, reduces interferences between new information and similar stored memories [Besnard and Sahay, 2016; Kheirbek et al., 2012]. This function is crucial to encode precise episodic memories and to further discriminate similar contextual representations. This mechanism permits to generate adaptive behaviors to our environment by allowing, for instance, the discrimination of contextual information previously associated with safety or danger. In animal studies, a reduced production of neurons in the dentate gyrus have been associated with pattern separation impairment [Clelland et al., 2009; Tronel et al., 2012]. Hence, the smaller dentate gyrus volumes reported in the PTSD group could reflect reduced pattern separation capacities compared to controls. Although we did not assess pattern separation abilities in this study, this hypothesis is consistent with several studies reporting impoverished autobiographical memories in adults with PTSD [Lapidow and Brown, 2015]. Indeed, this difficulty to recall specific and detailed episodes of their past (reported as overgeneralized memory) could be

partly caused by a low capacity to reduce the overlap between the memory traces of two similar experiences.

In addition to their implication in memory deficits, the CA2-3/DG alterations could be at the core of some psychopathological symptoms. Our study provides, for the first time in humans, evidence of a relation between specific hippocampal subfields alterations and intrusions in PTSD. Our results showed a negative correlation between the volume of the left CA2-3/DG region and the intrusion score in the PTSD group. As discussed previously, a reduced dentate gyrus volume could reflect low pattern separation capacities in the PTSD group. According to the theoretical model proposed by Besnard & Sahay [Besnard and Sahay, 2016], if the interferences between a new experience and the memory of the traumatic event are not resolved, non-related trauma cues could be recruited and associated with the memory trace of the traumatic event. This incorporation of non-related trauma cues could then lead to higher probabilities to reactivate the trauma memory in safe environments, and promote overgeneralization of fear [Besnard and Sahay, 2016]. Therefore, an alteration in the hippocampal subregion CA2-3/DG, known to be involved in pattern separation, could participate in the apparition of re-experiencing symptoms in adolescents with PTSD.

Methodological considerations and future directions

This study presents some methodological limitations. First, because of the cross-sectional design and the small size sample, we were not able to investigate the effects of trauma exposure and the development of PTSD on hippocampal maturation. The brain, and more precisely the hippocampus, undergoes several structural changes during adolescence. This plasticity is thought to be an adaptive response which allows adolescents to learn how to face new experiences without the care of their parents and, thus to be prepared for adulthood [Curlik et al., 2014]. However, despite this plasticity, some studies consider that the adolescent brain is more vulnerable to the effects of stress exposure than adults [Holder and

Blaustein, 2014; Romeo, 2017]. This hypothesis is based on evidence suggesting that the mechanisms involved in the stress response (such as the regulation of the hypothalamic-pituitary-adrenal axis and the neuro-inflammatory response) are less efficient in adolescents than in adults [Hueston et al., 2017]. In addition, a stress perceived during adolescence could negatively affect hippocampal maturation and cause negative long-term effects which will last into adulthood [Holder and Blaustein, 2014; Hueston et al., 2017]. Therefore, further studies are required to: (1) understand if the vulnerability to the effects of stress exposure on the brain during adolescence can be a risk factor to develop PTSD; and (2) investigate the effects of the development of PTSD on the maturation of hippocampal subfields. Second, we had a majority of adolescent females in the PTSD group. This can be explained by the fact that the prevalence of developing PTSD is higher in females than males [McLaughlin et al., 2013]. As a consequence, we were unable to assess if there was an effect of sex on hippocampal subfield alterations in adolescent PTSD. Nonetheless, studies in adults reported no significant hippocampal differences between females and males suffering from PTSD and thus, suggest that the hippocampal alterations are independent of sex [Woon and Hedges, 2011]. In addition, we conducted analyses on females only and we did not observe any difference with previous analyses performed on the whole group.

Conclusions

Adolescent PTSD was associated with hippocampal alterations. Using high resolution images and manual segmentation, we found smaller volume of the whole hippocampus (in both hemispheres) in the PTSD group. This first result highlights the importance of using segmentation methods adapted to an adolescent population to characterize brain differences in PTSD. Moreover, the hippocampal alterations were specific to CA2-3/DG and, the volume of this region was negatively correlated with intrusion symptoms. Hence, our study provides, for the first time in humans, evidence of a relation between specific hippocampal subfields

alterations and re-experiencing symptoms in PTSD. These findings resonate with recent theoretical models [Besnard and Sahay, 2016; Liberzon and Abelson, 2016] which propose that a reduced pattern separation (function subtended by CA3 and the dentate gyrus) could be at the core of the intrusions symptoms.

References

- American Psychiatric Association (2013): Diagnostic and statistical manual of mental disorders (5th ed.). Washington, D.C.: American Psychiatric Publishing.
- Berron D, Schütze H, Maass A, Cardenas-Blanco A, Kuijf HJ, Kumaran D, Düzel E (2016): Strong Evidence for Pattern Separation in Human Dentate Gyrus. *J Neurosci* 36:7569–7579.
- Besnard A, Sahay A (2016): Adult Hippocampal Neurogenesis, Fear Generalization, and Stress. *Neuropsychopharmacology* 41:24–44.
- Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, Rosoklija GB, Stankov A, Arango V, Dwork AJ, Hen R, Mann JJ (2018): Human Hippocampal Neurogenesis Persists throughout Aging. *Cell Stem Cell* 22:589-599.e5.
- Brunet A, St-Hilaire A, Jehel L, King S (2003): Validation of a French Version of the Impact of Event Scale-Revised. *Can J Psychiatry* 48:56–61.
- Carr VA, Rissman J, Wagner AD (2010): Imaging the human medial temporal lobe with high-resolution fMRI. *Neuron* 65:298–308.
- Clelland C, Choi M, Romberg C, Clemenson G, Fragniere A, Tyers P, Jessberger S, Saksida L, Barker R, Gage F, Bussey T (2009): A Functional Role for Adult Hippocampal Neurogenesis in Spatial Pattern Separation. *Science* 325:210–213.
- Curlik DM, DiFeo G, Shors TJ (2014): Preparing for adulthood: thousands upon thousands of new cells are born in the hippocampus during puberty, and most survive with effortful learning. *Front Neurosci* 8. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4005956/>.
- Daugherty AM, Flinn R, Ofen N (2017): Hippocampal CA3-dentate gyrus volume uniquely linked to improvement in associative memory from childhood to adulthood. *NeuroImage* 153:75–85.
- Dégeilh F, Viard A, Guénoles F, Gaubert M, Egler P-J, Egret S, Gerardin P, Baleyte J-M, Eustache F, Dayana J, Guillery-Girard B (2017): Functional brain alterations during self-reference processing in adolescents with sexual abuse-related post-traumatic stress disorder: A preliminary report. *Neurocase* 23:52–59.
- Dugas M, Bouvard M (1996): French version of the children depression inventory. In: Guelfi, J, editor. *L'évaluation clinique standardisée en psychiatrie*. Boulogne: Pierre Fabre. pp 543–548.
- Duvernoy H (2005): *The Human Hippocampus: Functional Anatomy, Vascularization and Serial Sections with MRI*. Berlin: Springer-Verlag.
- Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn A-M, Nordborg C, Peterson DA, Gage FH (1998): Neurogenesis in the adult human hippocampus. *Nat Med* 4:1313–1317.
- First M, Spitzer R, Gibbon M, Williams J (1996): *Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV)*. Washington, D.C.: American Psychiatric Press, Inc.
- de Flores R, la Joie R, Landeau B, Perrotin A, Mézenge F, de la Sayette V, Eustache F, Desgranges B, Chételat G (2015): Effects of age and Alzheimer's disease on hippocampal subfields. *Hum Brain Mapp* 36:463–474.
- Gilbertson MW, Shenton ME, Ciszewski A, Kasai K, Lasko NB, Orr SP, Pitman RK (2002): Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat Neurosci* 5:1242–1247.
- Gogtay N, Nugent TF, Herman DH, Ordóñez A, Greenstein D, Hayashi KM, Clasen L, Toga AW, Giedd JN, Rapoport JL, Thompson PM (2006): Dynamic mapping of normal human hippocampal development. *Hippocampus* 16:664–672.
- Gould E (2007): Structural plasticity. In: Andersen, P, Morris, R, Amaral, D, Bliss, T, O'Keefe, J, editors. *The hippocampus book*. New York: Oxford University Press. pp 321–341.

- Heine VM, Maslam S, Zareno J, Joëls M, Lucassen PJ (2004): Suppressed proliferation and apoptotic changes in the rat dentate gyrus after acute and chronic stress are reversible. *Eur J Neurosci* 19:131–144.
- Hill AS, Sahay A, Hen R (2015): Increasing Adult Hippocampal Neurogenesis is Sufficient to Reduce Anxiety and Depression-Like Behaviors. *Neuropsychopharmacology* 40:2368–2378.
- Holder MK, Blaustein JD (2014): Puberty and Adolescence as a Time of Vulnerability to Stressors that Alter Neurobehavioral Processes. *Front Neuroendocrinol* 35:89–110.
- Horner AJ, Doeller CF (2017): Plasticity of hippocampal memories in humans. *Curr Opin Neurobiol* 43:102–109.
- Huang Y, Coupland NJ, Lebel RM, Carter R, Seres P, Wilman AH, Malykhin NV (2013): Structural Changes in Hippocampal Subfields in Major Depressive Disorder: A High-Field Magnetic Resonance Imaging Study. *Biol Psychiatry* 74. Sources of Treatment Resistance in Depression: Inflammation and Functional Connectivity:62–68.
- Hueston CM, Cryan JF, Nolan YM (2017): Stress and adolescent hippocampal neurogenesis: diet and exercise as cognitive modulators. *Transl Psychiatry* 7:e1081.
- Keding TJ, Herringa RJ (2015): Abnormal Structure of Fear Circuitry in Pediatric Post-Traumatic Stress Disorder. *Neuropsychopharmacology* 40:537–545.
- Kempermann G (2008): The neurogenic reserve hypothesis: what is adult hippocampal neurogenesis good for? *Trends Neurosci* 31:163–169.
- Kheirbek MA, Klemenhagen KC, Sahay A, Hen R (2012): Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. *Nat Neurosci* 15:1613–1620.
- Kovacs M (1981): Rating scales to assess depression in school-aged children. *Acta Paedopsychiatr* 46:305–315.
- La Joie R, Fouquet M, Mézenge F, Landeau B, Villain N, Mevel K, Pélerin A, Eustache F, Desgranges B, Chételat G (2010): Differential effect of age on hippocampal subfields assessed using a new high-resolution 3T MR sequence. *NeuroImage* 53:506–514.
- Lapidow E, Brown A (2015): Autobiographical memories and PTSD. In: Martin, C, Preedy, V, Patel, V, editors. *Comprehensive Guide to Post-Traumatic Stress Disorders*. Basel: Springer. pp 1–13.
- Lee JK, Ekstrom AD, Ghetti S (2014): Volume of hippocampal subfields and episodic memory in childhood and adolescence. *NeuroImage* 94:162–171.
- Liberzon I, Abelson JL (2016): Context Processing and the Neurobiology of Post-Traumatic Stress Disorder. *Neuron* 92:14–30.
- Lobbestael J, Leurgans M, Arntz A (2011): Inter-rater reliability of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID I) and Axis II Disorders (SCID II). *Clin Psychol Psychother* 18:75–79.
- Logue MW, van Rooij SJH, Dennis EL, Davis SL, Hayes JP, Stevens JS, Densmore M, Haswell CC, Ipser J, Koch SBJ, Korgaonkar M, Lebois LAM, Peverill M, Baker JT, Boedhoe PSW, Frijling JL, Gruber SA, Harpaz-Rotem I, Jahanshad N, Koopowitz S, Levy I, Nawijn L, O'Connor L, Olf M, Salat DH, Sheridan MA, Spielberg JM, van Zuiden M, Wintemitz SR, Wolff JD, Wolf EJ, Wang X, Wrocklage K, Abdallah CG, Bryant RA, Geuze E, Jovanovic T, Kaufman ML, King AP, Krystal JH, Lagopoulos J, Bennett M, Lanius R, Liberzon I, McGlinchey RE, McLaughlin KA, Milberg WP, Miller MW, Ressler KJ, Veltman DJ, Stein DJ, Thomaes K, Thompson PM, Morey RA (2018): Smaller Hippocampal Volume in Posttraumatic Stress Disorder: A Multisite ENIGMA-PGC Study: Subcortical Volumetry Results From Posttraumatic Stress Disorder Consortia. *Biol Psychiatry* 83:244–253.
- Maren S, Phan KL, Liberzon I (2013): The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci* 14:417–428.

- McLaughlin KA, Koenen KC, Hill ED, Petukhova M, Sampson NA, Zaslavsky AM, Kessler RC (2013): Trauma Exposure and Posttraumatic Stress Disorder in a National Sample of Adolescents. *J Am Acad Child Adolesc Psychiatry* 52:815-830.e14.
- Morey RA, Haswell CC, Hooper SR, De Bellis MD (2016): Amygdala, Hippocampus, and Ventral Medial Prefrontal Cortex Volumes Differ in Maltreated Youth with and without Chronic Posttraumatic Stress Disorder. *Neuropsychopharmacology* 41:791–801.
- Mutluer T, Şar V, Kose-Demiray Ç, Arslan H, Tamer S, Inal S, Kaçar AŞ (2017): Lateralization of Neurobiological Response in Adolescents with Post-Traumatic Stress Disorder Related to Severe Childhood Sexual Abuse: the Tri-Modal Reaction (T-MR) Model of Protection. *J Trauma Dissociation* 19:108–125.
- Pitman RK, Rasmusson AM, Koenen KC, Shin LM, Orr SP, Gilbertson MW, Milad MR, Liberzon I (2012): Biological Studies of Posttraumatic Stress Disorder. *Nat Rev Neurosci* 13:769–787.
- Rolls ET (2016): Pattern separation, completion, and categorisation in the hippocampus and neocortex. *Neurobiol Learn Mem* 129. Pattern Separation and Pattern Completion in the Hippocampal System:4–28.
- Romeo RD (2017): The impact of stress on the structure of the adolescent brain: Implications for adolescent mental health. *Brain Res* 1654. Adolescence as a critical period for developmental plasticity:185–191.
- Schoemaker D, Buss C, Head K, Sandman CA, Davis EP, Chakravarty MM, Gauthier S, Pruessner JC (2016): Hippocampus and amygdala volumes from magnetic resonance images in children: Assessing accuracy of FreeSurfer and FSL against manual segmentation. *NeuroImage* 129:1–14.
- Schoenfeld TJ, McCausland HC, Morris HD, Padmanaban V, Cameron HA (2017): Stress and Loss of Adult Neurogenesis Differentially Reduce Hippocampal Volume. *Biol Psychiatry* 82. Stress: Novel Mechanisms and Treatments:914–923.
- Small SA, Schobel SA, Buxton RB, Witter MP, Barnes CA (2011): A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nat Rev Neurosci* 12:585–601.
- Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Boström E, Westerlund I, Vial C, Buchholz BA, Possnert G, Mash DC, Druid H, Frisén J (2013): Dynamics of hippocampal neurogenesis in adult humans. *Cell* 153:1219–1227.
- Tronel S, Belnoue L, Grosjean N, Revest J-M, Piazza P-V, Koehl M, Abrous DN (2012): Adult-born neurons are necessary for extended contextual discrimination. *Hippocampus* 22:292–298.
- Wang Z, Neylan TC, Mueller SG, Lenoci M, Truran D, Marmar CR, Weiner MW, Schuff N (2010): Magnetic Resonance Imaging of Hippocampal Subfields in Posttraumatic Stress Disorder. *Arch Gen Psychiatry* 67:296–303.
- Weiss D, Marma C (1997): The Impact of Event Scale-Revised. In: Wilson, J, Keane, T, editors. *Assessing psychological trauma and PTSD: a practitioner's handbook*. New York City: Guilford Press. pp 399–411.
- Woon F, Hedges DW (2011): Gender does not moderate hippocampal volume deficits in adults with posttraumatic stress disorder: A meta-analysis. *Hippocampus* 21:243–252.
- Woon FL, Hedges DW (2008): Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: A meta-analysis. *Hippocampus* 18:729–736.

Table 1: Demographic and psychopathological measures, depicting means and standard deviations (SD), for adolescents with PTSD and healthy controls

	PTSD Mean \pm SD	Controls Mean \pm SD	t	p-value
Number (F/M)	15 (13/2)	24 (12/12)	-	-
Age (months)	187.20 \pm 18.46	196.92 \pm 21.08	1.46	0.151
IQ (WISC-IV) [§]	97.43 \pm 19.48	109.04 \pm 17.24	1.91	0.064
Index trauma (n)	Sexual abuse (11) Witness of suicide (2) Car accident (1) Loss of loved one (1)	-	-	-
PTSD duration (months)	25.27 \pm 24.58	-	-	-
Age onset (years)	13.33 \pm 1.72	-	-	-
IES-R				
Intrusion	20.40 \pm 8.58	8.16 \pm 7.29	4.76	<0.001
Hyperarousal	13.60 \pm 5.37	3.21 \pm 4.83	6.26	<0.001
Avoidance	18.07 \pm 7.10	8.63 \pm 6.99	4.07	<0.001
CDI	19.73 \pm 9.58	9.17 \pm 4.85	4.57	<0.001

[§]Missing data of one patient

Table 2: TIV-adjusted hippocampal volumes in adolescents with PTSD and healthy controls

	TIV-adjusted Volume (mm ³)		GLM (PTSD effect)		
	PTSD	Controls	F	Uncorrected p	FDR-corrected p
Whole Hippocampus					
Left	3011 ± 413	3511 ± 379	7.90	0.013	0.04
Right	3001 ± 373	3573 ± 342	13.78	0.0006	0.006
CA1					
Left	878 ± 152	966 ± 112	2.25	0.143	0.198
Right	877 ± 114	1001 ± 153	3.95	0.055	0.09
CA2-3/DG					
Left	857 ± 162	1083 ± 153	7.90	0.008	0.04
Right	890 ± 180	1068 ± 162	6.31	0.017	0.04
Subiculum					
Left	773 ± 164	841 ± 111	1.12	0.297	0.297
Right	830 ± 156	899 ± 112	2.09	0.158	0.198
Tail					
Left	503 ± 77	621 ± 250	1.54	0.223	0.248
Right	404 ± 133	605 ± 228	4.88	0.034	0.068

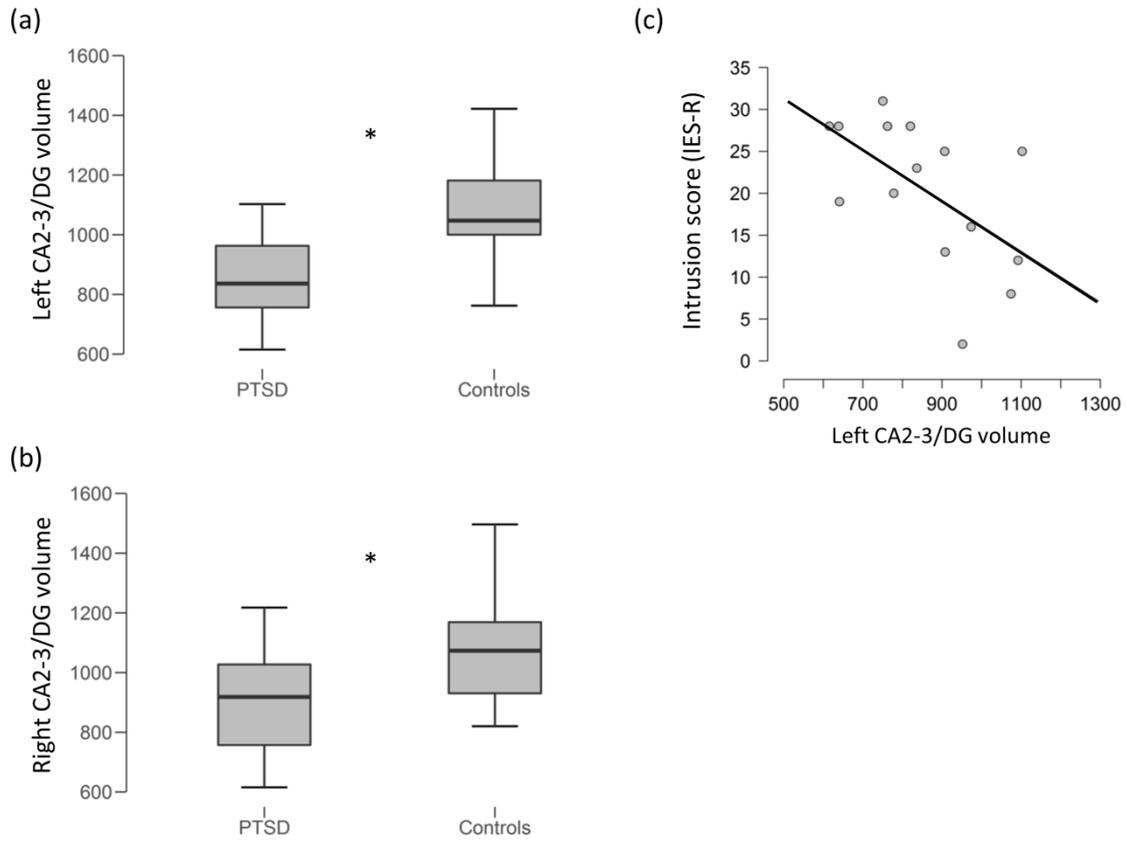


Figure 1 : (A, B) Significant differences (* $p_{FDR} < 0.05$) in TIV-adjusted CA2-3/DG volumes (mm³) between the PTSD group and the healthy controls; (C): Negative correlation between the TIV-adjusted left CA2-3/DG volume (mm³) and the intrusion score (IES-R) in the PTSD group