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STRUCTURAL BRAIN ALTERATIONS IN OLDER ADULTS EXPOSED TO EARLY-LIFE ADVERSITY

Marie-Laure Ancelin^{a*}, Isabelle Carriere^a, Sylvaine Artero^b, Jerome J Maller^{c,d,e}, Chantal Meslin^d, Anne-Marie Dupuy^{a,f}, Karen Ritchie^{a,g}, Joanne Ryan^{h,1}, Isabelle Chaudieu^{a,1}.

^a INM, Univ Montpellier, INSERM, Montpellier, France

^b IGF, Univ Montpellier, CNRS, INSERM, Montpellier, France

^c Monash Alfred Psychiatry Research Centre, Central Clinical School, Monash University and the Alfred Hospital, Melbourne, VIC, Australia

^d Centre for Mental Health Research, Australian National University, Canberra, Australia

^e General Electric Healthcare, Australia

^f INM, Univ Montpellier, INSERM, CHU Montpellier, Montpellier, France

^g Center for Clinical Brain Sciences, University of Edinburgh, UK

^h Biological Neuropsychiatry Unit, School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC, Australia

* Corresponding author: M-L Ancelin. Institute for Neurosciences of Montpellier, Inserm U1298, Hopital Saint-Eloi, 80, rue Augustin Fliche, BP 74103, 34091 Montpellier Cedex 5, France.

Tel: +33 499 614 562; Fax: +33 499 614 579; marie-laure.ancelin@inserm.fr

¹Joint last authors.

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ABSTRACT

Background: Adverse childhood events may have differential effects on the brain that persist into adulthood. Findings on structural brain alterations in older adults exposed to early-life adversity are inconsistent notably due to heterogeneity in imaging studies, population, psychiatric comorbidities, nature of adverse events, and genetic vulnerability. This study examines whether exposure related to physical or sexual maltreatment, emotional maltreatment, and global adverse environment during childhood are associated with specific alterations in grey matter volumes and if this varies according to sex and serotonin transporter-linked promoter region (*5-HTTLPR*) genotype.

Method: Structural MRI was used to acquire anatomical scans from 398 community-dwelling older adults. Quantitative regional estimates of 23 subregional volumes were derived using FreeSurfer software. Retrospective reporting of childhood adversity was collected using structured self-reported questionnaire. Analyses adjusted for age, sex, brain volume, head injury, lifetime depression and anxiety disorder, psychiatric medication, and cardiovascular ischemic pathologies.

Results: Exposure to adverse family environment was associated with smaller volumes of several frontal, cingulate, and parietal subregions and larger amygdala in the *5-HTTLPR SS* genotype participants specifically but larger volumes of caudate, putamen, pallidum, and nucleus accumbens in the *SL* genotype participants. Highly significant differences were found with excessive sharing of parent problems with children, associated with larger grey-matter volumes in the thalamus and several frontal and parietal regions in *5-HTTLPR SL* male participants specifically.

Conclusions: Early-life adversity is associated with grey-matter volume alterations in older adults and this varies according to the type of adversity experienced, sex, and serotonergic genetic vulnerability; *5-HTTLPR SS* participants appearing most vulnerable and *SL* individuals most resilient.

Keywords: Aging; cohort; sex; grey matter volume; childhood adversity; resilience.

1. Introduction

Although the link between childhood trauma and psychopathology is compelling, the long-term neurobiological signature resulting from childhood trauma is less well defined (Clausen et al., 2019; Teicher et al., 2016). Neurobiological models of childhood trauma suggest alterations on multiple levels, including changes in neurotransmission, neuroendocrine regulation (e.g. via the hypothalamic-pituitary-adrenal (HPA) stress axis) and in regional brain development (Lupien et al., 2009; McEwen, 2004). However, previous imaging studies have focused on only a few brain regions in young/middle aged adults. Little is known about the extent and persistence of structural changes in later life especially in the general older population.

The most consistent report of abnormalities resulting from childhood trauma has been grey matter volume (GMV) alterations in dorsolateral prefrontal, anterior cingulate and orbitofrontal cortex, with no strong evidence regarding the hippocampus and amygdala (see for meta-analyses and reviews (Calem et al., 2017; Cassiers et al., 2018; Paquola et al., 2016; Teicher and Samson, 2016)). Conversely, structural alterations in temporal, parietal and striatal regions have received little attention although these regions have been implicated in emotional processing and memory retrieval and suppression, which in turn are commonly altered in people exposed to trauma (Brewin, 2011; Gilmore et al., 2015; Kuhn and Gallinat, 2013; Li et al., 2014). Childhood trauma has generally been linked with decreased volume within these regions, but there is some indication that age, sex, psychiatric comorbidity, and genetic vulnerability may impact the robustness or directionality of these findings (Clausen et al., 2019; Popovic et al., 2020; Teicher and Samson, 2016). Inconsistencies may also be related to study design and size, population heterogeneity, type of trauma, and methodological issues, including insufficient data or power to examine confounding factors.

Research on the long term consequences of childhood trauma has mostly adopted a cumulative model of psychological impact, assuming severity of morbidity will be principally associated with cumulative exposures. This model has assumed all events to have similar underlying etiological mechanisms, and thus precluded the exploration of the impact of different types of

trauma on the central nervous system. More recently Sheridan et al. have proposed a pathology model (dimensional model of adversity and psychopathology, DMAP) which differentiates events characterized by deprivation and threat; the former compromising subsequent cognitive processing, and the latter emotional reactivity and autonomic regulation (Sheridan et al., 2020). Both dimensions may also differ regarding associated neural substrates (Sheridan and McLaughlin, 2020). The model is, however, hypothesis driven and while substantiating evidence exists from cluster analyses, research on the relationship between brain structures and different types of trauma is also needed.

Serotonergic genetic vulnerability, modifying neurotransmission and stress response, could render some individuals more susceptible to the impact of early trauma (Brouwer et al., 2017; Logue et al., 2015). Neuroimaging studies suggest increased serotonin synthesis in multiple brain regions in post-traumatic stress disorder (PTSD), with lower serotonin transporter (5-HTT) availability and correlations between 5-HTT and PTSD symptom severity (Davis et al., 2017). The *SLC6A4* gene encoding this transporter contains a polymorphism (*5-HTTLPR*), which consists of a 44 bp insertion/deletion referred to as long (L) and short (S) allele respectively, the latter being associated with reduced 5-HTT activity and serotonin reuptake. Two meta-analyses reported a significantly increased risk of PTSD with the *SS* genotype in cohort studies of patients with PTSD and/or individuals having experienced severe trauma (Gressier et al., 2013; Navarro-Mateu et al., 2013). However, despite some evidence for altered serotonergic function following trauma and a pivotal role of *5-HTTLPR* in emotional learning processes, its implication in the association between structural brain alterations and childhood adversity has rarely been examined in non-psychiatric older adults.

There is some evidence that sex can moderate the relationship between early adverse experiences and structural brain changes (Popovic et al., 2020; Tiwari and Gonzalez, 2018; Wellman et al., 2018). Male children were reported to be more vulnerable to the consequences of adversity with accompanying structural modifications in brain structure (De Bellis et al., 2001; Teicher and Samson, 2016). Sex could also modulate the effects of serotonergic polymorphisms and their

interactions with environmental stress factors (Perry et al., 2017; Tiwari and Gonzalez, 2018). In a study of predictors of hippocampal volume an interaction was observed between sex, *5-HTTLPR* genotype, and severe childhood adversity, only male *S'*-allele carriers with severe childhood abuse having smaller hippocampi (Everaerd et al., 2012). Overall, few studies have examined sex differences and these have mostly focused on clinical samples of adolescents or young adults and examined a limited number of brain regions. Rarely has genetic vulnerability been considered (Cassiers et al., 2018; Popovic et al., 2020).

To address the limitations of previous studies, we investigated the association between early-life adversity and frontal–subcortical and limbic subregions in a large, community-dwelling older population. We tested the hypothesis that older adults exposed to early-life adversity (total and specific event types) would have reduced GMV in several regional brain structures and this may vary according to the nature of the childhood event. We further hypothesized that this could differ according to sex and *5-HTTLPR* genetic variability, males and/or *SS* participants being the most susceptible to brain structural alterations.

2. METHODS

2.1. Participants

Data were obtained from a longitudinal study of neuropsychiatric disorders in community-dwelling French older adults, the ESPRIT study (Ritchie et al., 2004). Eligible participants, who were at least 65 years of age and non-institutionalized, were recruited by random selection from the electoral rolls of the Montpellier district between 1999 and 2001. Ethics approval for the study was given by the Ethical Committee of the University Hospital of Kremlin-Bicêtre and written informed consent was obtained. Of the 1863 participants initially recruited, only those aged 80 years or younger were invited for an MRI; 760 participants were randomly selected of whom 668 had complete volumetric data. Excluded were participants diagnosed with dementia ($n=14$), individuals

who were left-handed ($n=16$), and participants with missing data related to the genotyping ($n=81$), childhood adversity questionnaire ($n=106$) or covariates ($n=53$). Compared to the excluded participants, the 398 participants were younger ($p=0.0002$) and less likely to have low education level ($p=0.04$) and cognitive impairment ($p=0.01$).

2.2. MRI protocol and image analysis

All the neuroimaging scans were acquired using the same scanner and analyzed as described previously (Ancelin et al., 2019). Briefly, a 1.5T GE Signa Imaging system (General Electric Medical Systems, Milwaukee, WI) was used to acquire a contiguous AC-PC aligned axial IR-prepared SPGR T1-weighted sequence for volumetric estimations (TR=12, TE=2.8, IT=6000, matrix, size=256x256, pixel spacing=0.9375x0.9375mm, NEX=1, slice thickness=1.0mm). Regional reconstruction and segmentation was performed with the FreeSurfer 5.3 image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>). Twenty-three regions of interest (ROIs) defined using Desikan's Atlas (Desikan et al., 2006) were selected based on our previous studies in older adults (Ancelin et al., 2020; Ancelin et al., 2019) and literature on the structural brain alterations associated with early-life adverse events (see for reviews (Calem et al., 2017; Cassiers et al., 2018; Paquola et al., 2016; Teicher and Samson, 2016)). Total brain volume (grey+white matter) was computed for each participant using the segment m-file of the SPM5 software (Wellcome Department of Cognitive Neurology, UK), SPM showing greater accuracy (Malone et al., 2015) and consistency (Sargolzaei et al., 2015a) and less systematic bias evaluation (Sargolzaei et al., 2015b) than FreeSurfer for this measure.

2.3. Childhood adversity questionnaire

A self-administered questionnaire based on a review of existing validated instruments examined environment during childhood and adolescence (Ritchie et al., 2009). It contained items

corresponding to various adverse experiences with binary yes/no response categories and was completed at the second follow-up assessment, 4 years after recruitment. By this time the study interviewers had established close relationships with the participants, facilitating the request of sensitive information. Eight types of childhood traumas were examined; sexual or physical abuse or excessive physical punishment; neglect; serious conflict or stress at home; excessive sharing of parent problems; verbal abuse; poverty or financial difficulties; exposure to war or natural catastrophe, as well as a combined variable, adverse family environment variable. This variable included one of the following experiences: serious childhood illness, sent to a foster family, or having parents divorced or separated, hospitalized, with serious illness, prisoner for extended period, with alcohol or mental problems, suicidal or prematurely dead. The total number of childhood adversity experiences were also summed, and a three-category variable generated for the analysis.

2.4. Sociodemographic and clinical variables

A standardized interview was used to obtain information on socio-demographic characteristics, physical health, and medical history on cardiovascular ischemic pathologies (angina pectoris, myocardial infection, stroke, cardiovascular surgery, and arteritis). All drugs used in the preceding month were recorded from medical prescriptions and drug packaging. Weight and height were measured and body mass index was calculated. Global cognitive function was evaluated using the Mini-Mental State Examination, a score <26 indicating cognitive impairment (Folstein et al., 1975). Lifetime major depression and anxiety disorder (phobia, generalized anxiety disorder, panic disorder, obsessive compulsive disorder, as well as PTSD) were diagnosed by psychologists and psychiatric nurses according to DSM-IV criteria and using the Mini-International Neuropsychiatric Interview (MINI, French version 5.00), a standardized psychiatric examination validated in the general population (Sheehan et al., 1998).

2.5. 5-HTTLPR genotyping

We collected blood samples at baseline, enabling DNA extraction and 5-HTTLPR genotyping as described previously and data was validated using replicate independent genotyping of buccal DNA (Ancelin et al., 2019; Ancelin et al., 2017).

2.6. Statistical analysis

Associations between brain regions and exposure to early adverse events considered individually were evaluated using ANCOVA adjusted for age, sex, total brain volume, and covariates which could modify the associations, *e.g.* head injury, lifetime major depression and anxiety disorder, psychiatric medication, and cardiovascular ischemic pathologies as described previously (Ancelin et al., 2019). Given the frequent report of heterosis for 5-HTTLPR due to multiple sources of interacting effect (*e.g.* epistasis, sex, childhood adversity) (Ancelin and Ryan, 2018), the modifying effect of 5-HTTLPR was evaluated by stratification into three genotypes (*LL*, *SL*, and *SS*) as described (Ancelin et al., 2019; Ancelin et al., 2017). To account for the multiple brain regions examined, we adjusted the significance levels in the overall analysis using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995). “Nominal” associations referred to significant associations before FDR correction. The correlations between early life events were analysed using tetrachoric correlation coefficient (Olsson, 1979). All tests were 2-sided—and SAS (v9.4, SAS Institute, Inc., NC) was used for the statistical analyses.

3. RESULTS

3.1. Participant characteristics

Baseline characteristics of the 398 community-dwelling participants are summarized in **Table 1**. Participants ranged in age from 65 to 80, with a median age of 70 years and 52% were women. One quarter of the participants had lifetime major depression and anxiety disorder, and 1% had

lifetime PTSD. Men and women differed across all characteristics examined, with the exception of PTSD.

3.2. Subregional volumes according to adverse experiences during childhood

Nearly 8% of the participants reported sexual/physical trauma during childhood, and this was associated with 5.1% larger inferior temporal volumes and 7.2% larger entorhinal volume in adjusted models (**Table 2**). These associations however, were not significant after FDR correction. No significant sex interaction was found ($p>0.20$).

Regarding emotional trauma, conflict at home during childhood was reported by 17.4% of the participants, excessive sharing of parent problems by 11.9%, verbal abuse by 6.6%, and neglect by 5.4%. Only a few nominal associations were found with GMV, namely 3.8% larger inferior temporal and 5% larger entorhinal volumes with conflict/stress at home, 5.9% larger superior temporal volume with verbal abuse and larger volumes of caudate (7.6%) and putamen (9.4%) with neglect, the latter surviving FDR correction (**Supplementary Table S1**). On the other hand, excessive sharing of problems was associated with around 4% larger GMV in several frontal and parietal regions as well as insula, middle temporal region, and thalamus (**Table 3**). Alterations in rostral middle frontal, lateral orbitofrontal, superior parietal, precuneus, and thalamus volumes survived FDR correction. There was some evidence of effect modification by sex and in stratified analyses, the associations were found to be significant in men only. Further adjusting separately for other health factors which appeared sex-specific (cf. Table 1) did not modify the associations (data not shown).

Exposure to global environment factors during childhood was frequent in this older adult population; 58.9% reported traumatic experience related to war or natural catastrophe, 55% adverse family environment, and 24.8% poverty or financial difficulties. All three were nominally associated with larger volumes in hippocampus (2.2 to 2.6%), amygdala (3.0 to 3.6%), and some basal nuclei; nucleus accumbens for war or poverty (4.8%), pallidum (3.1%) and putamen (3.7%) for adverse

family environment (**Supplementary Table S2**).

3.3. Co-occurrence and correlations between adversity types

Co-occurrence of adverse events was relatively frequent in this sample; 29.6% of the participants having reported two, 13.2% three, and 12.5% four or more adverse experiences. A summary three-category variable was created for the total number of childhood adversity experiences. A significant association was only observed with hippocampal and amygdala volumes (FDR p -values >0.01) but not with frontoparietal volumes. Findings were similar for the intermediate and highest exposure group, between sexes and according to *5-HTTLPR* genotype (data not shown).

In addition, we examined the possible correlation between childhood adverse events for which we found significant changes in brain volumes, namely excessive sharing of parent problems and adverse family environment. No significant correlation was found [tetrachoric correlation (Asymptomatic Standard Error) $r=0.17$ (0.10), $p=0.11$] (data not shown).

3.4. Subregional volumes according to *5-HTTLPR* genotype

Of all participants, 29.4% were homozygous carriers of the L allele, and 24.6% were SS homozygotes. The frequency of the *5-HTTLPR* genotypes did not deviate significantly from Hardy-Weinberg equilibrium ($p=0.12$). There was no significant differences in participant characteristics according to *5-HTTLPR* genotypes (**Supplementary Table S3**). Given that we found that adverse family environment and excessive sharing of parent problems were most strongly associated with GMV, we further examined whether the *5-HTTLPR* genotype influenced these associations (**Table 4**).

Different patterns emerged depending across the *5-HTTLPR* genotypes. For *SL* participants, adverse family environment was associated with larger volumes of basal nuclei, *e.g.* nucleus accumbens and pallidum (around 5%), and, surviving FDR correction, caudate and putamen (around 7%). Conversely, the *SS* participants with adverse family environment had smaller volumes of a

number of ROIs at nominal significance levels, especially in the frontal (superior, rostral middle, lateral orbitofrontal) and parietal (superior and precuneus) cortex (3.6 to 4.9%), as well as in rostral anterior cingulate (9.9%), but larger entorhinal cortex (12.1%) and amygdala volume (6.3%) (**Table 4A**). The latter was also observed in *LL* participants.

In terms of excessive sharing of parent problems, almost all associations were found in *SL* participants only, including significantly larger volumes in rostral middle frontal, lateral orbitofrontal, superior parietal, and precuneus volumes (5.8 to 7.7%, $p \leq 0.006$) (**Table 4B**). Nominal associations were also observed for superior frontal, inferior parietal, and insula, as well as middle temporal and thalamus volumes (+4.0 to 6.3%). Sex-specific analysis confirmed previously findings (Table 3) that these associations were specific to men with around 9% larger GMV and up to 11.3% for superior parietal cortex (**Supplementary Table S4**).

4. DISCUSSION

In this older community-dwelling population, early-life adversity was associated with widespread morphological brain differences and this varied according to the type of adversity, sex, and serotonergic genetic vulnerability. The most significant findings were seen in older adults who had experienced adverse events that may have required active child participation, e.g. parents sharing problems with their children and adverse family environment. Excessive sharing of parent problems was associated with larger GMV across a number of areas in the frontal, middle temporal, and parietal regions as well as the thalamus. Interestingly, stratified analyses showed that these associations were only significant in men. Furthermore, findings indicate that *SL* heterozygotes of the *5-HTTLPR* appeared the most “resilient” (larger GMV) in contrast with the *SS* homozygotes. Exposure to adverse family environment was nominally associated with smaller volumes of several frontal and parietal cortex subregions, as well as larger amygdala and entorhinal cortex in the *5-HTTLPR SS* participants, but larger striatum volumes in the *SL* participants. These findings were independent of

psychiatric history and psychiatric medication and quite distinct from alterations associated with lifetime major depression reported in this ESPRIT population (Ancelin et al., 2019). This suggests a specific enduring impact of early life adversity on brain structure rather than a link to psychiatric comorbidity. Together our findings are consistent with the general view that there may be types of exposure that can trigger adaptive or maladaptive responses to certain early-life adverse events (e.g. adverse family environment or managing situations beyond children maturity) and that genetic vulnerability may render some individuals more susceptible to the positive and negative impact of early experience (Teicher and Samson, 2016).

4.1. Influence of *5-HTTLPR* genotype

This study shows that the association between adverse experiences in early life and structural brain alterations in a non-clinical older population can be moderated negatively (*SS* genotype) but also positively (*SL*) according to serotonergic genetic vulnerability. Compensatory brain development or cognitive adaptation may be a tempting explanation (Gupta et al., 2017a; Popovic et al., 2020; Teicher et al., 2016), especially given that we have previously observed some adverse childhood events (e.g. physical/sexual or verbal abuse, parents with mental problems) are associated with better late-life cognitive functioning (Ritchie et al., 2011). Epigenetic modifications may also be involved. In adults, *SLC6A4* hypermethylation has been associated with psychopathology-related adversities, e.g. late-life depression, stress, and childhood maltreatment and the *5-HTTLPR* polymorphisms can have modifying effects on these association (Alexander et al., 2014; Cecil et al., 2020; Duman and Canli, 2015; Lam et al., 2018). Hence, many of the reported alterations make sense as potentially adaptive responses modulated by *5-HTTLPR* (Teicher and Samson, 2016). Whether this may represent a marker of resilience-conferring brain adaptations in response to early stressful life events remains to be explored.

Genes involved in monoaminergic, neurotrophic and stress systems are likely to be the primary candidates determining the interaction of early life environment on GM development (Paquola et al.,

2016). In particular, the link between serotonergic signaling and *5-HTTLPR* genotype and HPA axis functioning is well documented (Andrews and Matthews, 2004). Two meta-analyses reported a significantly increased risk of PTSD with the *SS* genotype when studies were restricted to cohorts of non-clinical participants and/or having experienced high trauma (Gressier et al., 2013; Navarro-Mateu et al., 2013). In the ESPRIT study, we have shown reduced GMV in certain prefrontal and parietal ROIs (but not basal nuclei, amygdala or hippocampus), specifically in *SS* participants having experienced severe trauma as young adults with intrusive reexperiencing of symptoms (Ancelin et al., 2020). We did not observe increased volumes in *SL* participants, regardless of the ROIs.

4.2. Early life adversity and GMV

The most robust findings of previous structural studies examining early life events concerned deficits of the prefrontal and cingulate cortices (see for meta-analysis and review (Cassiers et al., 2018; Paquola et al., 2016)), in particular for physical and emotional abuse (Cohen et al., 2006; Heim et al., 2013; Tomoda et al., 2009; van Harmelen et al., 2010), but with differential associations across other trauma subtypes (Cassiers et al., 2018; Paquola et al., 2016). A study comparing multiple subtypes found that emotional neglect and certain adverse family environments (loss of primary family member) was associated with a reduction of anterior cingulate cortex volume, but this association was not observed in relation to emotional, physical or sexual abuse (Cohen et al., 2006).

Two meta-analyses examined the impact of childhood adversity on the hippocampus and amygdala in middle-aged adults in non-clinical and general population samples (Calem et al., 2017; Paquola et al., 2016). Both reported a modest impact on the hippocampus, but varied according to age and type of trauma, as observed in our study. Smaller hippocampal volume has been reported for general childhood adverse experiences (Cassiers et al., 2018), but not others such as neglect (Paquola et al., 2016). Early exposure to certain adverse environments (depressed mother, institutionally reared children) may result in increased amygdala volume but later exposure, psychopathology or high levels of stress in adulthood can result in decreased volumes (Calem et al.,

2017; Paquola et al., 2016; Teicher and Samson, 2016). This could help explain variability in findings with other studies (Paquola et al., 2016; Teicher and Samson, 2016).

Few studies have examined basal nuclei, and those that have mainly focused on the caudate nucleus (Cassiers et al., 2018; Cohen et al., 2006). Recently, Clausen and colleagues identified 13 GM regions associated with childhood trauma severity in young adults using a machine-learning approach, including caudate, pallidum, insula, as well as superior, inferior, and orbital frontal regions, and regions within temporal and parietal lobes (Clausen et al., 2019). The contribution of various subtypes of childhood trauma was however, not examined.

4.3. Sex differences

We found that men were more susceptible to structural alterations in response to a specific adverse event (excessive sharing of parent problems). Furthermore, this was strongest for men who carried the *SL* genotype. Few neuroimaging studies have investigated sex effects directly (Gupta et al., 2017b), however Popovic et al. recently reported contrary volumetric changes in the prefrontal cortex, hippocampus, amygdala, and anterior cingulate cortex for young adult men and women following childhood trauma (Popovic et al., 2020). There is other evidence that male children appear more vulnerable to the consequences of adversity (De Bellis et al., 2001; Teicher and Samson, 2016), with early trauma associated with smaller GMV in the prefrontal cortex, amygdala, and hippocampus (Helpman et al., 2017). In addition, sex can modulate the effects of serotonergic polymorphisms and their interactions with environmental stress factors (Perry et al., 2017), and genetic risk factors may also affect neural plasticity related to stress response and threat processing in a sex-dependent manner (Helpman et al., 2017). A prior study found that severe childhood adversity (at least one adverse event) was associated with smaller hippocampal volume in male *5HTTLPR* *S'*-allele carriers only (Everaerd et al., 2012), but no other ROIs were examined. Whether sex or gender may influence

the nature of changes in some structures, be associated with specific processing strategies or reflect difference in resilience to some stressful situation, remains to be examined.

4.4. Context of the findings

Our findings show that structural brain alterations are still observed in non-psychiatric older adults who have experienced adverse childhood events more than 50 years earlier. Consistent with previous reports in younger adults (Paquola et al., 2016), we found several significant alterations in prefrontal/ cingulate cortex-limbic system, primary centres for fear processing, emotional regulation and stress response, and also playing an important role in the regulation of the HPA axis (Helpman et al., 2017; Lupien et al., 2009; McEwen, 2004). However, the effects of stress at different stages in life could depend on the brain areas that are developing at the time of the exposure. The hippocampus is most vulnerable before 2 years of age, the amygdala continues to develop from birth to late childhood, whereas prefrontal and precuneus are among the last regions to mature (Cavanna and Trimble, 2006; Lupien et al., 2009; Teicher and Samson, 2016). This may explain why we found weak evidence for associations with hippocampal volume.

We found differences in regions that were rarely examined before, reward-and-motivation-related striatal regions, insula implicated in interoceptive, salience and self-referential processing (Clausen et al., 2019; Helpman et al., 2017; Paquola et al., 2016), and the entorhinal cortex also involved in memory processes. The processing of highly emotional memories in the aftermath of traumatic experiences not only relies on prefrontal cortices, but also on the 'parietal memory network' involved in multiple stages of mnemonic processing during both initial encoding and later retrieval (Gilmore et al., 2015). The precuneus has been implicated in imagery and visualization of visuo-spatial information in perception and memory, familiarity, and self-representation (Summerfield et al., 2009). Greater GMV of parietal subregions has been associated with resilience in healthy young adults (Gupta et al., 2017a), aligning with our findings for 'resilient' *SL* participants. Interestingly, in the DMAP model the frontoparietal network was suggested as neural substrate for the deprivation

dimension, which refers to an absence of, or an age-inappropriate, cognitive/stimulating environment (Sheridan and McLaughlin, 2020). Dimensional and cumulative models of adversity are not mutually exclusive and our findings also suggest that it is not just the number of experiences but also the type of experience that may have the greatest effect.

4.5. Limitations and strengths

This study has several limitations; the most important is the healthy survivor effect (survival bias) which may lead to a selection of the less vulnerable participants (Johnson et al., 2020) and subsequently an underestimation of some associations. Furthermore, the study has been conducted using self-report questionnaire and retrospective measures of childhood maltreatment which may identify different groups of individuals compared with prospective measures as reported in young adults (Baldwin et al., 2019). To minimize recall bias, the participants with probable/possible dementia were excluded. For some types of trauma (e.g. physical and sexual abuse), low numbers precluded definitive conclusions. We did not assess age at which the childhood trauma occurred and the cross-sectional design limits the ability to assess temporal relationships. Although this study adjusted for a number of potential confounders, we could not excluded that the more pronounced effects in men could be related to residual sex-specific confounding. Finally, multiple analyses have been performed potentially increasing the risk of type 1 error, although we have attempted to minimize this by correcting for multiple comparisons. Likewise, some experiences were rare resulting in insufficient power to detect differences in some stratified analyses.

However, this is one of the largest structural MRI studies targeting early-life events in older adults, in terms of the number of participants and ROIs examined within a single study and the first one to consider also *5-HTTLPR*. Further strengths are that data were collected on a wide range of childhood adverse events at a time by which the clinical staff had established trusting relationships with the participants. Brain volumes were measured by FreeSurfer automated segmentation, enabling accurate evaluation of volumetric changes of smaller deep brain structures. Lastly, we

controlled for genotyping accuracy and several potential confounders, particularly psychiatric comorbidity, which was assessed using a standardized psychiatric examination according to DSM-IV criteria.

5. Conclusions

In conclusion, these findings suggest long-term effects of childhood adverse events on the brain in older adults. The GMV pattern associated with early life adversity overlaps with brain networks of emotional processing and regulation, memory, and fear as well as the parietal memory network also shown to mediate introspection and resilience (Admon et al., 2013; Etkin and Wager, 2007; Gilmore et al., 2015; Gupta et al., 2017a; Hayes et al., 2012; Shin and Liberzon, 2010). According to serotonergic genetic vulnerability, these alterations may reflect both protective adaptation of and damage to the brain following exposure to threatening life events. These findings need to be replicated in larger population and clinical samples. It remains to be determined whether GMV increases are linked to a neuronal adaptive compensatory process in response to dysfunction in other structures, or a consequence of learned behaviors or skills, or whether they represent trait-like, developmental differences (which may be influenced by (epi)genetic factors) that underlie a neurobiological vulnerability associated with etiological pathways. Longitudinal studies examining the functional impact of structural changes in this broader network of regions are needed to clarify the role each may play in longer-term outcomes following trauma.

Contributors

M-L.A designed the study. M-L.A and K.R. lead the ESPRIT study and the collection of data. J.M. and C.M. processed all neuroimaging data. I.Ca. performed statistical analysis. A.M.D. performed DNA extraction and *5-HTTLPR* genotyping. M-L.A , S.A., J.R., and I.Ch. were involved in the interpretation of the data. M-L.A drafted the manuscript and all authors were involved in its revision and gave final approval to the submitted manuscript.

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Competing interest's statement

The authors declare no conflicts of interest.

Data availability statement

Any requests for data can be sent to the corresponding author.

Additional information

Supplementary information accompanies this paper (3 Supplementary Tables).

ORCID: Marie-Laure Ancelin <http://orcid.org/0000-0002-1149-4320>

References

- Admon, R., Milad, M.R., Hendler, T., 2013. A causal model of post-traumatic stress disorder: disentangling predisposed from acquired neural abnormalities. *Trends Cogn Sci* 17, 337-47.
- Alexander, N., Wankerl, M., Hennig, J., Miller, R., Zänkert, S., Steudte-Schmiedgen, S., Stalder, T., Kirschbaum, C., 2014. DNA methylation profiles within the serotonin transporter gene moderate the association of 5-HTTLPR and cortisol stress reactivity. *Transl Psychiatry* 4, e443.
- Ancelin, M.L., Carriere, I., Artero, S., Maller, J.J., Meslin, C., Dupuy, A.M., Ritchie, K., Ryan, J., Chaudieu, I., 2020. Structural brain changes with lifetime trauma and re-experiencing symptoms is 5-HTTLPR genotype-dependent. *Eur J Psychotraumatol* 11, 1733247.
- Ancelin, M.L., Carriere, I., Artero, S., Maller, J.J., Meslin, C., Ritchie, K., Ryan, J., Chaudieu, I., 2019. Lifetime major depression and grey matter volume. *J Psychiatry Neurosci* 44, 45-53.
- Ancelin, M.L., Ryan, J., 2018. 5-HTTLPR x stress hypothesis: is the debate over? *Mol Psychiatry* 23, 2116-17.
- Ancelin, M.L., Scali, J., Norton, J., Ritchie, K., Dupuy, A.M., Chaudieu, I., Ryan, J., 2017. Heterogeneity in HPA axis dysregulation and serotonergic vulnerability to depression. *Psychoneuroendocrinology* 77, 90-4.
- Andrews, M.H., Matthews, S.G., 2004. Programming of the hypothalamo-pituitary-adrenal axis: serotonergic involvement. *Stress* 7, 15-27.
- Baldwin, J.R., Reuben, A., Newbury, J.B., Danese, A., 2019. Agreement Between Prospective and Retrospective Measures of Childhood Maltreatment: A Systematic Review and Meta-analysis. *JAMA Psychiatry* 76, 584-93.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc B* 57, 289-300.
- Brewin, C.R., 2011. The nature and significance of memory disturbance in posttraumatic stress disorder. *Annu Rev Clin Psychol* 7, 203-27.

- Brouwer, R.M., Panizzon, M.S., Glahn, D.C., Hibar, D.P., Hua, X., Jahanshad, N., Abramovic, L., de Zubicaray, G.I., Franz, C.E., Hansell, N.K., Hickie, I.B., Koenis, M.M.G., Martin, N.G., Mather, K.A., McMahon, K.L., Schnack, H.G., Strike, L.T., Swagerman, S.C., Thalamuthu, A., Wen, W., Gilmore, J.H., Gogtay, N., Kahn, R.S., Sachdev, P.S., Wright, M.J., Boomsma, D.I., Kremen, W.S., Thompson, P.M., Hulshoff Pol, H.E., 2017. Genetic influences on individual differences in longitudinal changes in global and subcortical brain volumes: Results of the ENIGMA plasticity working group. *Hum Brain Mapp* 38, 4444-58.
- Calem, M., Bromis, K., McGuire, P., Morgan, C., Kempton, M.J., 2017. Meta-analysis of associations between childhood adversity and hippocampus and amygdala volume in non-clinical and general population samples. *Neuroimage Clin* 14, 471-9.
- Cassiers, L.L.M., Sabbe, B.G.C., Schmaal, L., Veltman, D.J., Penninx, B., Van Den Eede, F., 2018. Structural and Functional Brain Abnormalities Associated With Exposure to Different Childhood Trauma Subtypes: A Systematic Review of Neuroimaging Findings. *Front Psychiatry* 9, 329.
- Cavanna, A.E., Trimble, M.R., 2006. The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 129, 564-83.
- Cecil, C.A.M., Zhang, Y., Nolte, T., 2020. Childhood maltreatment and DNA methylation: A systematic review. *Neurosci Biobehav Rev* 112, 392-409.
- Clausen, A.N., Aupperle, R.L., Yeh, H.W., Waller, D., Payne, J., Kuplicki, R., Akeman, E., Paulus, M., 2019. Machine Learning Analysis of the Relationships Between Gray Matter Volume and Childhood Trauma in a Transdiagnostic Community-Based Sample. *Biol Psychiatry Cogn Neurosci Neuroimaging* 4, 734-42.
- Cohen, R.A., Grieve, S., Hoth, K.F., Paul, R.H., Sweet, L., Tate, D., Gunstad, J., Stroud, L., McCaffery, J., Hitsman, B., Niaura, R., Clark, C.R., McFarlane, A., Bryant, R., Gordon, E., Williams, L.M., 2006. Early life stress and morphometry of the adult anterior cingulate cortex and caudate nuclei. *Biol Psychiatry* 59, 975-82.

- Davis, M.T., Holmes, S.E., Pietrzak, R.H., Esterlis, I., 2017. Neurobiology of chronic stress-related psychiatric disorders: evidence from molecular imaging studies. *Chronic Stress* 1, 1-21.
- De Bellis, M.D., Keshavan, M.S., Beers, S.R., Hall, J., Frustaci, K., Masalehdan, A., Noll, J., Boring, A.M., 2001. Sex differences in brain maturation during childhood and adolescence. *Cereb Cortex* 11, 552-7.
- Desikan, R.S., Segonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S., Killiany, R.J., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 31, 968-80.
- Duman, E.A., Canli, T., 2015. Influence of life stress, 5-HTTLPR genotype, and SLC6A4 methylation on gene expression and stress response in healthy Caucasian males. *Biol Mood Anxiety Disord* 5, 2.
- Etkin, A., Wager, T.D., 2007. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry* 164, 1476-88.
- Everaerd, D., Gerritsen, L., Rijpkema, M., Frodl, T., van Oostrom, I., Franke, B., Fernández, G., Tendolkar, I., 2012. Sex modulates the interactive effect of the serotonin transporter gene polymorphism and childhood adversity on hippocampal volume. *Neuropsychopharmacology* 37, 1848-55.
- Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12, 189-98.
- Gilmore, A.W., Nelson, S.M., McDermott, K.B., 2015. A parietal memory network revealed by multiple MRI methods. *Trends Cogn Sci* 19, 534-43.
- Gressier, F., Calati, R., Balestri, M., Marsano, A., Alberti, S., Antypa, N., Serretti, A., 2013. The 5-HTTLPR polymorphism and posttraumatic stress disorder: a meta-analysis. *J Trauma Stress* 26, 645-53.

- Gupta, A., Love, A., Kilpatrick, L.A., Labus, J.S., Bhatt, R., Chang, L., Tillisch, K., Naliboff, B., Mayer, E.A., 2017a. Morphological brain measures of cortico-limbic inhibition related to resilience. *J Neurosci Res* 95, 1760-75.
- Gupta, A., Mayer, E.A., Acosta, J.R., Hamadani, K., Torgerson, C., van Horn, J.D., Chang, L., Naliboff, B., Tillisch, K., Labus, J.S., 2017b. Early adverse life events are associated with altered brain network architecture in a sex- dependent manner. *Neurobiol Stress* 7, 16-26.
- Hayes, J.P., Hayes, S.M., Mikedis, A.M., 2012. Quantitative meta-analysis of neural activity in posttraumatic stress disorder. *Biol Mood Anxiety Disord* 2, 9.
- Heim, C.M., Mayberg, H.S., Mletzko, T., Nemeroff, C.B., Pruessner, J.C., 2013. Decreased cortical representation of genital somatosensory field after childhood sexual abuse. *Am J Psychiatry* 170, 616-23.
- Helpman, L., Zhu, X., Suarez-Jimenez, B., Lazarov, A., Monk, C., Neria, Y., 2017. Sex Differences in Trauma-Related Psychopathology: a Critical Review of Neuroimaging Literature (2014-2017). *Curr Psychiatry Rep* 19, 104.
- Johnson, J., Chaudieu, I., Ritchie, K., Scali, J., Ancelin, M.L., Ryan, J., 2020. The extent to which childhood adversity and recent stress influence all-cause mortality risk in older adults. *Psychoneuroendocrinology* 111, 104492.
- Kuhn, S., Gallinat, J., 2013. Gray matter correlates of posttraumatic stress disorder: a quantitative meta-analysis. *Biol Psychiatry* 73, 70-4.
- Lam, D., Ancelin, M.L., Ritchie, K., Freak-Poli, R., Saffery, R., Ryan, J., 2018. Genotype-dependent associations between serotonin transporter gene (SLC6A4) DNA methylation and late-life depression. *BMC Psychiatry* 18, 282.
- Li, L., Wu, M., Liao, Y., Ouyang, L., Du, M., Lei, D., Chen, L., Yao, L., Huang, X., Gong, Q., 2014. Grey matter reduction associated with posttraumatic stress disorder and traumatic stress. *Neurosci Biobehav Rev* 43, 163-72.

- Logue, M.W., Amstadter, A.B., Baker, D.G., Duncan, L., Koenen, K.C., Liberzon, I., Miller, M.W., Morey, R.A., Nievergelt, C.M., Ressler, K.J., Smith, A.K., Smoller, J.W., Stein, M.B., Sumner, J.A., Uddin, M., 2015. The Psychiatric Genomics Consortium Posttraumatic Stress Disorder Workgroup: Posttraumatic Stress Disorder Enters the Age of Large-Scale Genomic Collaboration. *Neuropsychopharmacology* 40, 2287-97.
- Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* 10, 434-45.
- Malone, I.B., Leung, K.K., Clegg, S., Barnes, J., Whitwell, J.L., Ashburner, J., Fox, N.C., Ridgway, G.R., 2015. Accurate automatic estimation of total intracranial volume: a nuisance variable with less nuisance. *Neuroimage* 104, 366-72.
- McEwen, B.S., 2004. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci* 1032, 1-7.
- Navarro-Mateu, F., Escamez, T., Koenen, K.C., Alonso, J., Sanchez-Meca, J., 2013. Meta-analyses of the 5-HTTLPR polymorphisms and post-traumatic stress disorder. *PLoS One* 8, e66227.
- Olsson, U., 1979. Maximum likelihood estimation of the polychoric correlation coefficient. *Psychometrika* 44, 443-60.
- Paquola, C., Bennett, M.R., Lagopoulos, J., 2016. Understanding heterogeneity in grey matter research of adults with childhood maltreatment-A meta-analysis and review. *Neurosci Biobehav Rev* 69, 299-312.
- Perry, L.M., Goldstein-Piekarski, A.N., Williams, L.M., 2017. Sex differences modulating serotonergic polymorphisms implicated in the mechanistic pathways of risk for depression and related disorders. *J Neurosci Res* 95, 737-62.
- Popovic, D., Ruef, A., Dwyer, D.B., Antonucci, L.A., Eder, J., Sanfelici, R., Kambeitz-Illankovic, L., Oztuerk, O.F., Dong, M.S., Paul, R., Paolini, M., Hedderich, D., Haidl, T., Kambeitz, J., Ruhrmann, S., Chisholm, K., Schultze-Lutter, F., Falkai, P., Pergola, G., Blasi, G., Bertolino, A., Lencer, R.,

- Dannlowski, U., Upthegrove, R., Salokangas, R.K.R., Pantelis, C., Meisenzahl, E., Wood, S.J., Brambilla, P., Borgwardt, S., Koutsouleris, N., 2020. Traces of Trauma: A Multivariate Pattern Analysis of Childhood Trauma, Brain Structure, and Clinical Phenotypes. *Biol Psychiatry* 88, 829-42.
- Ritchie, K., Artero, S., Beluche, I., Ancelin, M.L., Mann, A., Dupuy, A.M., Malafosse, A., Boulenger, J.P., 2004. Prevalence of DSM-IV psychiatric disorder in the French elderly population. *Br J Psychiatry* 184, 147-52.
- Ritchie, K., Jaussent, I., Stewart, R., Dupuy, A.M., Courtet, P., Ancelin, M.L., Malafosse, A., 2009. Association of adverse childhood environment and 5-HTTLPR genotype with late-life depression. *J Clin Psychiatry* 70, 1281-8.
- Ritchie, K., Jaussent, I., Stewart, R., Dupuy, A.M., Courtet, P., Malafosse, A., Ancelin, M.L., 2011. Adverse childhood environment and late-life cognitive functioning. *Int J Geriatr Psychiatry* 26, 503-10.
- Sargolzaei, S., Sargolzaei, A., Cabrerizo, M., Chen, G., Goryawala, M., Noei, S., Zhou, Q., Duara, R., Barker, W., Adjouadi, M., 2015a. A practical guideline for intracranial volume estimation in patients with Alzheimer's disease. *BMC Bioinformatics* 16 Suppl 7, S8.
- Sargolzaei, S., Sargolzaei, A., Cabrerizo, M., Chen, G., Goryawala, M., Pinzon-Ardila, A., Gonzalez-Arias, S.M., Adjouadi, M., 2015b. Estimating Intracranial Volume in Brain Research: An Evaluation of Methods. *Neuroinformatics* 13, 427-41.
- Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 59, 22-33.
- Sheridan, M.A., McLaughlin, K.A. 2020. Neurodevelopmental mechanisms linking ACEs with psychopathology. *Adverse Childhood Experiences*. Elsevier Inc, pp 265-85.

- Sheridan, M.A., Shi, F., Miller, A.B., Salhi, C., McLaughlin, K.A., 2020. Network structure reveals clusters of associations between childhood adversities and development outcomes. *Dev Sci* 23, e12934.
- Shin, L.M., Liberzon, I., 2010. The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology* 35, 169-91.
- Summerfield, J.J., Hassabis, D., Maguire, E.A., 2009. Cortical midline involvement in autobiographical memory. *Neuroimage* 44, 1188-200.
- Teicher, M.H., Samson, J.A., 2016. Annual Research Review: Enduring neurobiological effects of childhood abuse and neglect. *J Child Psychol Psychiatry* 57, 241-66.
- Teicher, M.H., Samson, J.A., Anderson, C.M., Ohashi, K., 2016. The effects of childhood maltreatment on brain structure, function and connectivity. *Nat Rev Neurosci* 17, 652-66.
- Tiwari, A., Gonzalez, A., 2018. Biological alterations affecting risk of adult psychopathology following childhood trauma: A review of sex differences. *Clin Psychol Rev* 66, 69-79.
- Tomoda, A., Suzuki, H., Rabi, K., Sheu, Y.S., Polcari, A., Teicher, M.H., 2009. Reduced prefrontal cortical gray matter volume in young adults exposed to harsh corporal punishment. *Neuroimage* 47 Suppl 2, T66-71.
- van Harmelen, A.L., van Tol, M.J., van der Wee, N.J., Veltman, D.J., Aleman, A., Spinhoven, P., van Buchem, M.A., Zitman, F.G., Penninx, B.W., Elzinga, B.M., 2010. Reduced medial prefrontal cortex volume in adults reporting childhood emotional maltreatment. *Biol Psychiatry* 68, 832-8.
- Wellman, C.L., Bangasser, D.A., Bollinger, J.L., Coutellier, L., Logrip, M.L., Moench, K.M., Urban, K.R., 2018. Sex Differences in Risk and Resilience: Stress Effects on the Neural Substrates of Emotion and Motivation. *J Neurosci* 38, 9423-32.

Table 1. Characteristics of the 398 participants.

	Whole sample (N=398)	Women (N=207)	Men (N=191)	p-value^a
	Median (IQR)			
Age, years	70 (68-74)	70 (67-73)	71 (68-74)	0.01
Body mass index, kg/m ² (n=397)	24.9 (22.7-27.0)	24.0 (21.5-26.3)	25.8 (24.2-27.7)	<0.0001
Cortex, cm ³	359 (338-385)	346 (324-366)	380 (355- 407)	<0.0001
Grey matter brain volume, cm ³	461 (415 – 503)	449 (393-482)	480 (429-525)	<0.0001
Total brain volume, cm ³	888 (822-965)	858 (793-910)	936 (862-1008)	<0.0001
	%			
Education level (≤ 5years)	22.6	25.1	19.9	0.21
Living alone	17.9	30.1	4.7	<0.0001
Head injury	10.6	5.8	15.7	0.001
Smoking				
Never	53.5	76.8	28.3	<0.0001
Past	39.2	17.9	62.3	
Current	7.3	5.3	9.4	
Lifetime major depression ^b	27.4	38.7	15.2	<0.0001
Lifetime anxiety disorder ^b	24.4	31.4	16.8	0.0007
Lifetime post-traumatic stress disorder ^b	1.0	1.0	1.1	0.94
Psychiatric medication (antidepressant or anxiolytic)	12.8	17.9	7.3	0.002
Hypertension (≥140/90 or treatment)	69.4	64.3	74.9	0.02
Cardiovascular ischemic pathology ^c	11.8	7.3	16.8	0.003
Diabetes ^d	8.6	3.9	13.7	0.0005
Cognitive impairment (MMSE score <26)	21.6	27.1	15.7	0.006

IQR = Interquartile range; MMSE = Mini-Mental State Examination.

^aSex differences were tested using Kruskal-Wallis tests for continuous variables and Chi-square tests for categorical variables.

^bDiagnosis of lifetime major depression or anxiety disorder (phobia, generalized anxiety disorder, panic disorder or obsessive compulsive disorder) according to DSM-IV criteria and using the MINI (Sheehan et al., 1998).

^cHistory of cardiovascular ischemic pathologies (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, arteritis).

^dFasting glucose ≥ 7.0 mmol/L or treatment.

Table 2. Grey matter volumes according to sexual/physical trauma during childhood (N=392, 30 events).

Brain regions	No ELE		ELE		p ^b
	Mean ^a	SEM ^a	Mean ^a	SEM ^a	
Superior frontal	34759	364	34779	605	0.972
Rostral middle frontal	24732	273	24676	454	0.897
Caudal middle frontal	9500	159	9437	263	0.804
Lateral orbitofrontal	11819	127	11842	211	0.911
Medial orbitofrontal	9130	113	8893	188	0.191
Rostral anterior cingulate	3444	73	3416	121	0.807
Caudal anterior cingulate	3082	68	3118	113	0.737
Posterior cingulate	5005	73	5014	121	0.943
Superior parietal	20581	262	21323	435	0.078
Inferior parietal	20620	273	20881	453	0.551
Precuneus	15036	169	15211	281	0.519
Insula	11908	128	12074	212	0.419
Superior temporal	17651	226	18071	375	0.247
Middle temporal	17720	229	17395	380	0.375
Inferior temporal	16815	243	17680	403	0.027
Entorhinal	3498	73	3751	121	0.031
Hippocampus	6933	86	6851	143	0.551
Amygdala	2629	40	2559	66	0.274
Thalamus	11575	115	11389	191	0.313
Caudate	6857	125	7091	207	0.242
Nucleus accumbens	965	18	956	31	0.768
Putamen	9238	131	9338	217	0.634
Pallidum	2950	41	3059	68	0.102

ELE= Early life event.

^aValues expressed in mm³ and adjusted for age, sex, total brain volume, head injury, lifetime major depression and anxiety disorder, psychiatric medication, and cardiovascular ischaemic pathologies.

^bRaw p values not adjusted for multiple comparisons.

Table 3. Grey matter volumes in men and women according to item 'excessive sharing of parent problems with child'.

Brain regions	Whole sample (N=387, 46 events)					Men (N=187, 21 events)					Women (N=200, 25 events)				
	No ELE		ELE		p ^b	No ELE		ELE		p ^b	No ELE		ELE		p ^b
Mean ^a	SEM ^a	Mean ^a	SEM ^a	Mean ^a		SEM ^a	Mean ^a	SEM ^a	Mean ^a		SEM ^a	Mean ^a	SEM ^a	Mean ^a	
Superior frontal	34574	369	35538	528	0.045	36036	555	37749	852	0.021	33184	635	33537	791	0.581
Rostral middle frontal	24515	274	25586	392	0.003*	25735	402	27299	617	0.004*	23713	482	24407	601	0.154
Caudal middle frontal	9470	160	9638	229	0.418	9915	229	10627	351	0.020	9447	288	9142	359	0.293
Lateral orbitofrontal	11729	128	12166	183	0.009*	12236	186	13014	286	0.002*	11298	228	11463	284	0.473
Medial orbitofrontal	9017	114	9349	163	0.025	9476	175	9809	269	0.153	8521	192	8814	240	0.131
Rostral anterior cingulate	3444	74	3467	106	0.811	3634	121	3542	186	0.569	3225	113	3310	141	0.451
Caudal anterior cingulate	3066	69	3160	98	0.293	3156	104	3201	159	0.743	2996	120	3125	150	0.284
Posterior cingulate	4979	74	5138	105	0.097	5267	112	5481	172	0.153	4723	126	4862	157	0.275
Superior parietal	20563	264	21508	377	0.006*	21105	400	22588	614	0.006*	20172	453	20579	565	0.372
Inferior parietal	20632	275	21170	392	0.132	21327	394	22056	604	0.164	20259	495	20579	617	0.521
Precuneus	14984	170	15622	243	0.004*	15622	265	16514	407	0.012*	14247	278	14632	347	0.171
Insula	11909	129	12218	185	0.065	12525	192	12962	295	0.087	11246	227	11395	283	0.515
Superior temporal	17646	229	17919	328	0.358	18294	311	18729	477	0.293	17518	437	17645	544	0.772
Middle temporal	17573	231	18259	331	0.023	18244	312	19679	478	0.0006*	17289	436	17215	543	0.865
Inferior temporal	16912	248	17157	354	0.446	17746	376	18269	577	0.295	16205	427	16152	532	0.902
Entorhinal	3502	74	3668	106	0.086	3840	115	3913	176	0.631	3184	121	3440	151	0.037
Hippocampus	6887	87	7022	125	0.234	7069	131	7066	200	0.985	6748	153	6977	191	0.138
Amygdala	2614	41	2671	58	0.272	2744	64	2788	99	0.604	2458	65	2510	81	0.424
Thalamus	11486	115	11959	165	0.002*	12074	178	12729	273	0.006*	10873	189	11185	235	0.102
Caudate	6913	127	6967	182	0.742	6884	203	6996	312	0.679	6708	198	6633	246	0.709
Nucleus accumbens	962	19	985	27	0.350	1003	28	1018	43	0.684	906	33	936	41	0.356
Putamen	9268	131	9394	188	0.460	9470	194	9582	298	0.663	8824	232	8895	289	0.762
Pallidum	2969	41	3016	59	0.386	3095	65	3054	99	0.634	2839	68	2948	85	0.116

ELE = Early life event.

^aValues expressed in mm³ and adjusted for age, sex (whole sample), total brain volume, head injury, lifetime major depression and anxiety disorder, psychiatric medication, and cardiovascular ischaemic pathologies.

^bRaw p values not adjusted for multiple comparisons. *Indicates remaining significant even after FDR correction.

Table 4. Association of cortical ROI volumes with early-life events according to *5-HTTLPR* genotype.**4A. Adverse family environment**

	SS, n=98 (49 events)					SL, n=182 (103 events)					LL, n=116 (66 events)				
	No ELE		ELE			No ELE		ELE			No ELE		ELE		
Brain regions	Mean ^a	SEM ^a	Mean ^a	SEM ^a	p ^b	Mean ^a	SEM ^a	Mean ^a	SEM ^a	p ^b	Mean ^a	SEM ^a	Mean ^a	SEM ^a	p ^b
Superior frontal	35543	767	34161	666	0.028	34373	679	34960	574	0.248	34958	737	35512	750	0.301
Rostral middle frontal	25098	602	24121	523	0.047	24359	485	24324	410	0.922	24674	558	25401	568	0.075
Caudal middle frontal	9479	350	9108	304	0.193	9719	280	9817	237	0.642	9074	344	8964	350	0.659
Lateral orbitofrontal	12010	258	11522	224	0.021	11647	230	11742	195	0.582	11945	271	11934	276	0.953
Medial orbitofrontal	8962	254	8901	221	0.766	9207	198	9244	168	0.800	8841	249	8780	253	0.736
Rostral anterior cingulate	3598	163	3243	141	0.008	3449	122	3464	103	0.866	3570	176	3585	179	0.906
Caudal anterior cingulate	2993	162	2891	141	0.440	3246	109	3201	92	0.580	3011	161	3019	164	0.943
Posterior cingulate	4801	169	4794	147	0.954	5099	125	5118	106	0.839	4961	167	4997	170	0.763
Superior parietal	21160	559	20118	486	0.023	20294	484	20660	410	0.313	21209	549	20963	558	0.538
Inferior parietal	20738	641	20520	556	0.673	20502	492	20575	416	0.841	21334	557	21157	567	0.661
Precuneus	15371	355	14824	308	0.059	15075	311	15117	263	0.857	14970	342	15463	348	0.049
Insula	11731	288	11834	250	0.658	11619	233	11923	197	0.083	12473	270	12440	275	0.863
Superior temporal	17501	459	17345	399	0.675	17612	415	17917	352	0.327	17378	482	18017	491	0.070
Middle temporal	17744	478	17090	415	0.094	17321	425	17792	360	0.140	17902	456	18218	464	0.340
Inferior temporal	16733	570	17039	495	0.507	16798	423	17077	358	0.378	16732	502	17160	511	0.242
Entorhinal	3290	162	3689	141	0.003	3503	124	3490	105	0.886	3575	169	3666	172	0.455
Hippocampus	6728	178	6903	155	0.228	7007	149	7064	126	0.606	6670	203	6948	207	0.061
Amygdala	2478	93	2635	80	0.039	2655	70	2663	59	0.883	2513	85	2650	86	0.028
Thalamus	11306	249	11467	217	0.426	11820	198	11828	167	0.958	11238	271	11273	276	0.859
Caudate	6770	232	6579	202	0.310	6815	231	7330	195	0.003*	6605	277	6669	282	0.750
Nucleus accumbens	886	44	882	39	0.926	947	31	998	26	0.029	1028	41	972	41	0.060
Putamen	8713	274	8769	238	0.798	8993	215	9621	182	0.0001*	9570	297	9757	302	0.387
Pallidum	2815	100	2883	87	0.398	2953	70	3087	60	0.012	2945	85	3014	87	0.261

ELE = Early life event.

^aValues expressed in mm³ and adjusted for age, sex, total brain volume, head injury, lifetime major depression and anxiety disorder, psychiatric medication, and cardiovascular ischaemic pathologies.^bRaw p values. *Indicates remaining significant even after FDR correction.

4B. Excessive sharing of parent problems with child

	SS, n=94 (9 events)					SL, n=180 (25 events)					LL, n=113 (12 events)				
	No ELE		ELE		p ^b	No ELE		ELE		p ^b	No ELE		ELE		p ^b
Brain regions	Mean ^a	SEM ^a	Mean ^a	SEM ^a		Mean ^a	SEM ^a	Mean ^a	SEM ^a		Mean ^a	SEM ^a	Mean ^a	SEM ^a	
Superior frontal	34530	678	35097	1093	0.580	34190	581	35829	775	0.022	35070	749	35388	1091	0.712
Rostral middle frontal	24270	513	25470	827	0.124	23943	417	25352	557	0.006*	24897	578	25044	842	0.825
Caudal middle frontal	9268	297	9196	478	0.873	9652	243	10010	325	0.229	8950	348	8776	508	0.664
Lateral orbitofrontal	11655	227	11670	367	0.966	11531	198	12220	264	0.005*	11902	275	12129	401	0.473
Medial orbitofrontal	8812	208	9372	335	0.076	9137	173	9391	231	0.231	8788	252	9031	367	0.401
Rostral anterior cingulate	3325	143	3555	231	0.287	3381	105	3540	141	0.219	3592	172	3246	251	0.082
Caudal anterior cingulate	2878	137	3208	220	0.112	3165	95	3346	127	0.119	3072	161	2806	234	0.151
Posterior cingulate	4800	145	4794	234	0.976	5036	109	5273	145	0.075	4977	166	5076	242	0.604
Superior parietal	20403	484	20626	781	0.760	20172	414	21848	552	0.001*	21098	552	21323	805	0.724
Inferior parietal	20638	538	20406	867	0.775	20113	417	21128	557	0.048	21173	569	21192	829	0.978
Precuneus	14925	307	15506	494	0.211	14841	266	15842	356	0.002*	15167	349	15302	508	0.735
Insula	11741	231	12023	373	0.420	11615	199	12169	265	0.024	12466	275	12476	401	0.975
Superior temporal	17515	395	16904	637	0.307	17619	361	18062	482	0.316	17657	496	18133	722	0.405
Middle temporal	17463	416	16508	670	0.131	17383	370	18396	494	0.026	18028	456	19294	665	0.017
Inferior temporal	16999	489	16660	788	0.646	16901	369	17456	492	0.220	16936	510	17049	744	0.847
Entorhinal	3556	147	3622	237	0.769	3477	106	3672	142	0.134	3535	171	3750	249	0.275
Hippocampus	6878	154	6804	248	0.752	6994	129	7051	173	0.717	6782	209	7179	305	0.101
Amygdala	2598	83	2529	133	0.583	2616	60	2737	80	0.101	2592	89	2596	130	0.964
Thalamus	11414	216	11521	348	0.744	11646	168	12134	225	0.019	11327	272	11977	396	0.039
Caudate	6679	203	6472	327	0.498	7048	202	7255	269	0.402	6736	283	6639	413	0.766
Nucleus accumbens	886	39	870	62	0.777	984	27	1021	36	0.263	1007	43	1020	62	0.785
Putamen	8825	227	8564	366	0.447	9386	196	9598	262	0.376	9772	302	10046	439	0.430
Pallidum	2894	82	2787	133	0.389	3042	63	3104	84	0.416	2983	87	3111	127	0.205

ELE = Early life event.

^aValues expressed in mm³ and adjusted for age, sex, total brain volume, head injury, lifetime major depression and anxiety disorder, psychiatric medication, and cardiovascular ischaemic pathologies.

^bRaw p values. *Indicates remaining significant even after FDR correction.