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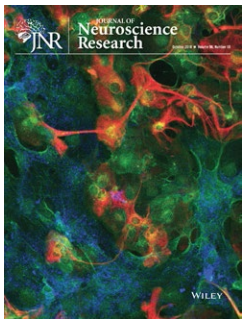
RESEARCH ARTICLE



Larger bilateral amygdalar volumes are associated with affective loss experiences

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Abstract

Affective loss (AL) (i.e., bereavement, relationship breakup) is a stressful life event leading to a heightened risk of developing a psychiatric disorder, for example, depression and anxiety disorder. These disorders have been associated with altered subcortical brain volumes. Little is known though, how AL in healthy subjects is linked to subcortical volumes. In a study with 196 healthy young adults, we probed the association between AL across the individual entire life span, assessed via the List of Threatening Experiences Questionnaire, and magnetic resonance imaging brain gray matter volumes (*a priori* selected: bilateral amygdalae, hippocampi, thalami; exploratory analyses: nuclei accumbens, caudate, putamina), segmented by use of volBrain. AL was defined as death of a first-degree relative/spouse, close relative/friend, and breakup of a marriage or steady relationship. AL was associated with larger bilateral amygdalar volumes and, after taking into account the total number of ALs, with smaller right hippocampal volumes, both irrespective of sex. Exploratory analyses of striatal volumes yielded an association of AL with larger right nucleus accumbens volumes in men, and increased caudate volumes after the loss of a first-degree relative irrespective of sex. Our data suggest that AL engenders alterations in limbic structures that likely involve processes of chronic stress and amygdala- and hippocampus-dependent fear conditioning, and resemble those observed in general anxiety disorder, childhood maltreatment, and major depressive disorder. Our exploratory findings of striatal volume alterations hint at a modulation of reward processing by AL.

KEYWORDS

anxiety, attachment, childhood maltreatment, depression, loss of loved one, MRI, stress, subcortical volumes

Significance

The risk of developing a major depressive or anxiety disorder is elevated after the experience of an affective loss (AL) such as bereavement or a relationship breakup. Subcortical alterations might underlie this association. Little is known though, how AL in healthy subjects is linked to subcortical volumes. With our study, we showed that AL engenders alterations in limbic brain

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volumes that likely involve processes of chronic stress and fear conditioning, and that were comparable to those observed in major depressive disorder and general anxiety disorder. Our findings further suggest that AL alters subcortical structures implicated in reward processing.

1 | INTRODUCTION

The loss of a loved one, due to bereavement or a romantic relationship dissolution, is a stressful, painful event (Boyle et al., 2011; Eisenberger, 2006; Tennant & Andrews, 1976). According to attachment theory, humans are innately motivated to form emotional bonds with significant others and to resist the disruption of these bonds "from cradle to grave" (Bowlby, 1969; Shear & Shair, 2005). Losing a loved one, in the following termed "affective loss," is often followed by transient, subclinical symptoms of anxiety and depression during the first weeks. However, long-lasting effects of affective loss (AL) on mental and physical health, such as an increased risk for major depressive disorder (MDD), generalized anxiety disorder (GAD), and for mortality, for up to 10 years after loss, have been observed (Biondi & Picardi, 1996; Boyle et al., 2011; Kendler et al., 1999, 2003; Monroe et al., 1999). Evidence is growing that AL can provoke physiological alterations in the immune system and in the stress axis that last as long as 18 to 24 months after bereavement, for instance increased adrenocortical activity (Biondi & Picardi, 1996; Hofer et al., 1972; Richardson et al., 2015), and there is some evidence that these alterations are modulated by individual risk factors, such as the personal history of other ALs (Biondi & Picardi, 1996). It has been proposed that AL might also engender long-lasting morphological and functional brain alterations which underlie the observed prolonged vulnerability to illness after AL (Biondi & Picardi, 1996).

To date, in one voxel-based morphometry study, the number of ALs within the past 5 years has been related to alterations in cerebellar gray matter volume, modulated by adult attachment style (Benetti et al., 2010), but findings have been inconsistent (Acosta et al., 2018). Investigating grief-related brain activations using functional magnetic resonance imaging, within 1 year (Gündel et al., 2003; Kersting et al., 2009; Najib et al., 2004) or within 5 years after AL (O'Connor et al., 2008), altered neural activations in the networks of pain and emotions (e.g., the cingulate, striatum, insula, thalamus, amygdala, among others) have been reported. Of note, different types of AL have been addressed by these studies which probed either the bereavement of a first-degree relative (Gündel et al., 2003; O'Connor et al., 2008), the loss of an unborn child (Kersting et al., 2009), the breakup of a relationship (Najib et al., 2004), or the bereavement of a close relative, a friend, and/or the separation from a spouse (Benetti et al., 2010). However, to date, the neural correlates of different types of AL have not yet been compared in a study. In sum, to the best of our knowledge, little is known about the effects of AL on subcortical brain structures.

Subcortical brain regions of sensory processing, emotion processing, and emotion regulation, such as amygdala, hippocampus, and thalamus, are engaged during psychogenic stress exposure (e.g., Ulrich-Lai & Herman, 2009). In animal studies, prolonged or chronic stress experiences have modified the physiology and structure of these brain regions in a partly irreversible manner, inducing amygdalar hypertrophy as well as hippocampal and thalamic atrophy (Magariños et al., 1996; Vyas et al., 2002, 2004; Yoshii et al., 2017). The factors that influence the reversibility of chronic stress effects on brain structure are still unclear (Sousa, 2016). In human neuroimaging studies, enlarged amygdalar volumes have been associated with long-term occupational stress (Savic, 2015). Reduced hippocampal volumes have been related to the number of general stressful life events during the last 3 months (Papagni et al., 2011), higher perceived life stress during the past 20 years (Gianaros et al., 2007), childhood maltreatment (Berens et al., 2017; Hart & Rubia, 2012), and post-traumatic stress disorder (Bremner, 1999; Karl et al., 2006).

There is growing evidence that stress exposure also affects reward processing (Ulrich-Lai & Herman, 2009) and decision-making (Dias-Ferreira et al., 2009) which both rely on activity in dorsal and ventral striatal regions, such as the nucleus accumbens (ventral), caudate and putamen (both dorsal). The striatum contains receptors for neurochemical mediators of stress (Novick et al., 2018). Stress exposure has increased spine density in the nucleus accumbens of mice (Warren et al., 2014), and stress-related anhedonia has been associated with dendritic hypertrophy of neurons in the rat nucleus accumbens (Bessa et al., 2013). Chronic stress has also been related to structural changes in the rat dorsal striatum paralleling a behavioral bias toward habit (Dias-Ferreira et al., 2009). In human neuroimaging studies, reduced dorsal striatal volumes have been associated with long-term occupational stress (Blix et al., 2013; Savic, 2015) and early life stress (Cohen et al., 2006), and enlarged nucleus accumbens volumes mediated the link between peer problems and adolescent depression (Lee et al., 2020).

In summary, chronic stress exposure affects subcortical structures that are also altered in anxiety and depression (e.g., Drevets et al., 2008; Duval et al., 2015). Anxiety and depression have been linked to enlarged amygdalar volumes (De Bellis et al., 2000; Frodl et al., 2002, 2003; Schienle et al., 2011), and to reduced hippocampal and thalamic volumes (Arnone et al., 2012; Hettema et al., 2012; McKinnon et al., 2009; Moon et al., 2015; Nugent et al., 2013). There is also some evidence that anxiety and depression are associated with increased nucleus accumbens volumes (Günther et al., 2018; Kühn et al., 2011; Lee et al., 2020), and with dorsal striatal volume alterations (i.e., reductions in MDD (Ancelin et al., 2019; Bora et al., 2012) and enlargements in GAD (Hilbert et al., 2015; Liao et al., 2013, 2014)). However, hardly anything is known about the effects of AL on subcortical structures in healthy subjects.

With this cross-sectional study in healthy young adults, we wanted to probe the association between AL experiences across

the individual entire life span and subcortical volumes that are implicated in the stress response. We selected the amygdala, hippocampus, and thalamus as *a priori* regions of interest. We expected larger amygdalar gray matter volumes, but smaller hippocampal and thalamic volumes in individuals with AL compared to those without AL. Given the growing evidence for an overlap between stress and reward processing, we aimed at exploring the association of AL with volumes of the nucleus accumbens and the dorsal striatum, namely caudate and putamen. We expected larger nucleus accumbens volumes in individuals with AL compared to those without AL. No specific hypotheses were generated for dorsal striatal volumes given the opposing effects of chronic stress exposure, MDD, and GAD on these volumes. We defined AL as death of a first-degree relative or a spouse, close relative and/or close friend, separation from a spouse, and breakup of a steady relationship occurring at any time from birth to the time of investigation. Sex-specific effects were explored given the sexually dimorphic mortality risk after bereavement (Biondi & Picardi, 1996), sex-specific associations of AL with cerebral brain structures (Acosta et al., 2018), and the high density of sex steroid receptors in the investigated subcortical structures (Goldstein et al., 2001). Furthermore, we wanted to explore how the different types of AL are associated with subcortical volumes, considering that different types of AL could differently modulate subcortical structures. In additional exploratory analyses, we aimed at testing whether the elapsed time after AL and the total number of AL are associated with subcortical volumes, considering the approach of other studies which investigated AL experiences only in a limited time interval after AL (e.g., Gündel et al., 2003) and/or as a sum of AL experiences (Benetti et al., 2010). To complement our region-of-interest (ROI) analyses, we additionally performed a whole-brain voxel-based morphometry (VBM) analysis. While our ROI analyses predicated upon label-fusion-based segmentation which results in a cumulative volumetric measure, our whole-brain VBM analysis was based on voxel-by-voxel measurements. Segmentation and voxel-by-voxel VBM analyses provide different types of information on brain structural alterations and it has been suggested that they should be used in tandem (Giuliani et al., 2005). In our study, we will focus on the segmented brain volumes, taking into account that VBM is more prone to registration errors and multiple comparison problems (Grimm et al., 2015) and that its accuracy varies between subcortical structures (Focke et al., 2014).

2 | METHODS

2.1 | Participants

Neuroimaging data from 198 healthy subjects were collected. Inclusion criteria were student status, age (18–40 years), right-handedness (as assessed by the Edinburgh Inventory, Oldfield, 1971, inclusion criterion > +40), German as native language, and Western- or Middle-European descent. Exclusion criteria were history of major psychiatric disorders of participants and their first-degree relatives according to ICD-10 (assessed by the Mini-International Neuropsychiatric Interview, Ackenheil et al., 1999), relevant medical or neurological diseases, psychology students (to avoid a bias due to familiarity with a study task manipulation which is of no relevance here), and metal implants or other magnetic resonance imaging (MRI) contraindications. All participants were students at the Universities of Marburg or Gießen (Germany). All individual participants gave written informed consent, and the study protocol was approved by the local ethics committee according to the Declaration of Helsinki. Two subjects were excluded from the analysis of structural data because of low data quality due to motion artifacts. The characteristics of the remaining 196 subjects included into the analyses were as follows: 50% women; mean age = 24.0 years, *SD* = 3.2, median = 23.0 years, range 19–38. The age distribution in our sample (4.1% < 20 years old, 88.8% 20–29 years old, 7.1% 30–39 years old) matched the typical age distribution of German students.

2.2 | Measures and procedure

All questionnaires were administered prior to scanning (in general at least 1 day beforehand).

2.2.1 | Affective loss

We assessed the number of ALs using a subset of questions from the List of Threatening Experiences Questionnaire (LTE-Q) (Brugha & Cragg, 1990). The LTE-Q is a self-report questionnaire consisting of 12 items. For the purposes of our study, we selected items 3, 4, 5, and 6 of the LTE-Q to measure AL experiences that constituted the loss of a first-degree relative or spouse, loss of a close friend or close relative, separation from a spouse, and breakup of a steady relationship. Similarly, in the study of Benetti et al. (2010), probing the association of AL with brain structure, the LTE-Q items 3, 4, and 5 had been selected. We additionally included the breakup of a steady relationship (item 6) as there is evidence that the breakup of a steady relationship is experienced as an AL and increases the risk for poor mental health (Najib et al., 2004; Sbarra, 2006).

AL experience was assessed as subjects' age in yearly intervals which defined the accuracy of the assessment of the elapsed time after the last AL (see 2.2.6.3 b). We computed a dichotomous AL variable dividing the sample into subjects with no AL versus those with at least one AL, occurring at any time from birth to the time of investigation (AL = 0: *N* = 42, vs. AL > 0: *N* = 154). In order to control for possible modulatory effects of the individual number of previous AL (Benetti et al., 2010; Biondi & Picardi, 1996), we included the total number of AL into our analyses (*N* = 188, missings: *N* = 8). To elucidate whether brain structure is differently affected by the occurrence of AL in childhood/adolescence (<18 years) and/or adulthood (≥18 years), we created a factor with four levels (AL-CAA, missings:

N = 7): no AL (*N* = 42), AL only in childhood/adolescence (*N* = 33), AL only in adulthood (*N* = 68), and AL in both childhood/adolescence and adulthood (*N* = 46). Missings of this variable were due to imprecise or lacking information about the timing of the loss(es).

2.2.2 | Control variables

To control for general anxiety, we administered the State-Trait Anxiety Inventory, Trait version (STAI-T; Laux et al., 1981; Spielberger et al., 1970) (Cronbach's $\alpha = 0.90$). The Beck Depression Inventory (BDI; Beck & Steer, 1987; Hautzinger et al., 1994) was used to measure depressive symptoms (Cronbach's $\alpha = 0.61$). To control for potentially confounding effects of attachment security in childhood, it was assessed with the German version of the Hazan-Shaver scale (Hazan & Shaver, published in Collins & Read, 1990; Neumann, 2002). Participants reporting a secure attachment to both parents were coded as secure (*n* = 105), all others were coded as insecure (for details see Schneider-Hassloff et al., 2016). Further, we assessed the separation or divorce of parents until the subject's age of 18. Childhood maltreatment has been related to altered subcortical volumes (Frodl et al., 2017; Opel et al., 2014), and we assessed childhood maltreatment with the Childhood Trauma Screener (CTS) (Grabe et al., 2012). The CTS contains five items measuring emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. We used the sum score of all CTS items (CTS Sum) and additionally the CTS item score of emotional neglect in our control analyses, reasoning that especially emotional neglect might be experienced as chronic/repeated AL.

2.2.3 | Acquisition of MR images

Data were acquired on a 3 Tesla whole-body scanner (Siemens MAGNETOM Trio– A Tim System, Germany) at the Department of Psychiatry and Psychotherapy, University of Marburg. A three-dimensional (3D) fast gradient echo sequence (GRAPPA) was used to acquire T1-weighted high-resolution anatomical images (repetition time = 1,900 ms, echo time = 2.52 ms, flip angle = 9°, long-term averages, inversion prepulse every 900 msec, field of view of 256 (feet–head [FH]) × 256 (anterior–posterior [AP]) × 176 (right–left [RL]) mm, phase encoding in AP and RL direction, voxel size = 1 × 1 × 1 mm).

2.2.4 | MRI preprocessing and brain structure segmentation for the region-of-interest analyses

The native anatomical images were preprocessed and segmented applying the volBrain pipeline (Manjón & Coupé, 2016). The pipeline is based on the following steps: (a) Spatially adaptive nonlocal means denoising, (b) rough inhomogeneity correction, (c) affine registration to Montreal Neurological Institute (MNI) space, (d) fine Statistical Parametric Mapping (SPM)-based inhomogeneity correction, (e) intensity normalization, (f) nonlocal intracranial cavity extraction, (g) tissue classification, (h) nonlocal hemisphere segmentation, and (i) nonlocal subcortical structure segmentation. The subcortical structure segmentation was performed applying nonlocal label fusion (for details, please refer to Coupé et al., 2011; Manjón & Coupé, 2016). Image processing quality and segmentation were visually assessed for all subjects by using volBrain reports, and two subjects were excluded due to low image quality. We used the uncorrected volumes of left and right amygdalae, hippocampi, and thalami, as well as total intracranial volumes (TIVs) as provided by the volBrain segmentation in the statistical analyses (2.2.6). For the exploratory analyses, we used the uncorrected volumes of left and right nuclei accumbens, caudate, and putamina.

2.2.5 | MRI whole-brain analysis

To complement our region-of-interest analyses, we additionally performed a whole-brain voxel-based morphometry analysis. Structural images were preprocessed using CAT12 Toolbox (version 12.1 http://www.neuro.uni-jena.de/cat/) and SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) with standard routines and templates. Images were bias-corrected, tissue classified, and normalized into a standard stereotactic anatomical MNI space (resulting voxel size 1.5 × 1.5 × 1.5 mm), employing high-dimensional DARTEL normalization within a unified model (Ashburner, 2007; Ashburner & Friston, 2005). The homogeneity of the resulting modulated gray matter volumes was examined by means of a covariance matrix implemented in the check data quality function. Two outliers showing covariances below 0.855 were identified and excluded (identical with the excluded ones in the subcortical structure segmentation in 2.2.4). The modulated gray matter images were smoothed with a Gaussian kernel of 8 mm full width at half maximum (FWHM). SPM12 was used to calculate second-level group statistics. Localization of peaks is reported as MNI coordinates. For the anatomical localization of the structural data, probabilistic cytoarchitectonic maps according to the SPM Anatomy Toolbox (version 2.2c; http://www.fz-juelich.de/inm/inm-1/DE/Forschung/_docs/SPMAnatomyToolbox/SPMAnatomyToolbox_node.html) (Eickhoff et al., 2005) were used as reference.

2.2.6 | Statistical analyses

Statistical analyses of descriptive information and region-of-interest data (2.2.4) were performed using R 3.6.3 (http://www.r-project.org/) (R Core Team, 2016). Packages in use were “Hmisc” (Harrell, 2012), “nortest” (Gross & Ligges, 2015), “ggplot2” (Wickham, 2016), “car” (Fox & Weisberg, 2011), and “psych” (Revelle, 2019) among others. The alpha level of statistical significance was set at $p < 0.05$ for all analyses. In order to control for the error rate related to multiple comparisons, we additionally report a false-discovery-rate (FDR)

correction that was used for the six outcome measures (left/right amygdalae, left/right hippocampi, and left/right thalami) (p.adjust function in R). No correction for multiple testing was carried out in the exploratory analyses. Assumptions of the multiple regression analyses were checked by visual inspection of the correct specification of the model (Residuals vs. Fitted plot, Lowess line), the normal distribution of the residuals (Normal Q–Q plot), the homoscedasticity (Scale-Location diagram), and critical outliers (Residuals vs. Leverage plot, Cook's distance) ("plot" function in R). Assumptions were met in all analyses unless otherwise stated. Homoscedasticity was additionally tested by means of the Goldfeld–Quandt test; a p value > 0.05 indicates homoscedasticity. Heteroscedasticity was observed in analyses with left caudate volumes in the subsample, and a regression with robust standard errors, applying a heteroscedasticity-consistent covariance matrix estimation according to Halbert White (R package "Sandwich"; Zeileis, 2004; Zeileis et al., 2020), was additionally computed. The regressions with robust standard errors yielded very similar results (data not shown). The normality of the distribution of the outcome variables was checked with the Lilliefors (Kolmogorov–Smirnov) normality test. All outcome variables were normally distributed (all $p > 0.09$) except left putamen volumes ($p = 0.050$) in the whole sample, and right hippocampal volumes ($p = 0.026$) in the subsample (AL > 0). Given the large sample size (>50), the central limit theorem, and the Normal Q–Q plots, we expected no effects of nonnormality on the analyses with these volumes. However, we additionally computed the logarithm of left putamen and right hippocampal volumes which were normally distributed ($p = 0.527$/$p = 0.206$), and we repeated all related analyses: Only negligible changes in the results were observed (data not shown). To probe multicollinearity, we computed the variance inflation factor (VIF); a VIF < 10 is considered as an indicator of no multicollinearity. As for the descriptive information, group differences with regard to sex and AL were analyzed by use of t-tests or chi-squared tests, and associations between descriptive variables were assessed as the Pearson Product Moment correlation coefficient or in multiple regression analyses with brain volumes as outcome, controlling for age, sex, and TIV.

No AL versus at least one AL and subcortical structures

Standard multiple regression analyses were performed to probe the association of the dichotomized AL variable (AL = 0 vs. AL > 0) with left and right amygdalae, hippocampi, and thalami in independent analyses. Goldfeld–Quandt tests indicated homoscedasticity (all $p > 0.07$), and no multicollinearity was detected (all VIFs < 2). In exploratory analyses, we analyzed the association of the dichotomized AL variable with left and right nuclei accumbens, caudate, and putamina (Goldfeld–Quandt tests: all $p > 0.2$). All analyses included age, sex (women coded as 0 and men as 1) and TIVs as control variables. In order to probe whether the relation between AL and brain volumes differs between AL in childhood/adolescence and/or adulthood (see 2.2.1), we computed multiple regression analyses with the variable AL-CAA as predictor, sex, age, and TIV as covariates, and used the group with no AL as reference group, in independent analyses for each subcortical volume (Goldfeld–Quandt tests: all $p > 0.06$, all VIFs < 2). Furthermore, we explored sex differences by analyzing the interaction of the dichotomous AL variable with sex (Goldfeld–Quandt tests: all $p > 0.08$, all VIFs < 6).

We repeated the multiple regression analyses in order to control for clinical symptoms, attachment-related measures, childhood trauma, and the total number of AL, by additionally including following covariates in five independent control analyses: (a) depressive symptoms (BDI) and trait anxiety (STAI-T), (b) childhood attachment security and parental separation, (c) CTS Sum score, (d) CTS emotional neglect, and (e) total number of AL (AL: all VIFs < 2; AL × sex: all VIFs < 6; AL-CAA: all VIFs < 3).

Exploratory analysis: Different types of AL and subcortical structures

We explored whether the loss of a first-degree relative or spouse (AL-type 1; no/yes: $N = 179/17$), the loss of a close friend or close relative (AL-type 2; no/yes: $N = 93/103$), and the breakup of a steady relationship (AL-type 4; no/yes: $N = 98/98$) are differently related to subcortical volumes. We omitted the analysis of the separation from a spouse, because no subject in our sample reported this type of AL. We included all three variables as predictors into the multiple regression analyses in addition to age, sex, and TIV as control variables, and subcortical volumes as dependent variable (Goldfeld–Quandt tests: all $p > 0.1$, all VIFs < 2). Control analyses were performed as described above (all VIFs < 3.2).

Exploratory analyses: Subsample of subjects with at least one AL experience

Number of past AL and subcortical structures. We explored the association of the total number of AL with subcortical structures (Benetti et al., 2010; Biondi & Picardi, 1996; O'Connor et al., 2008). In the subsample of the subjects with at least one AL experience ($N = 154$), we probed the association of brain gray matter volumes with the total number of AL as predictor in independent multiple regression analyses. Sex, age, and TIV were included as covariates (Goldfeld–Quandt tests: all $p > 0.08$ except for left caudate: $p = 0.050$, all VIFs < 2, final sample size due to missings of the predictor: $N = 146$). We performed control analyses as described above (all VIFs < 2). Results are reported in SI. Significant results are also reported in the results section.

The elapsed time after the last AL and subcortical structures. Further, we explored linear and U-shaped associations between subcortical volumes and the time that had elapsed after the last reported AL experience (Goldfeld–Quandt tests: all $p > 0.06$ except for left caudate: $p = 0.028$, all VIFs < 2, final sample size due to missings of the predictor: $N = 148$). U-shaped associations were probed by quadratic regressions (Goldfeld–Quandt tests: all $p > 0.07$ except for left caudate: $p = 0.029$, all VIFs < 8) and by the two-lines test (Simonsohn, 2018). Results are reported in SI. Significant results are also reported in the results section.

Complementary analysis: Whole-brain analyses

In the whole-brain analysis, we analyzed the association of no AL versus at least one AL in a two-sample *t*-test, based on the general linear model (GLM), comparing the group of no AL with the group with at least one AL. We included sex, age, and TIV as covariates. We chose a significance threshold of $p < 0.05$ FWE (family-wise error rate) corrected, but we also report results with an uncorrected threshold of $p < 0.001$ and a minimum cluster size of 50 voxels. Results are reported in SI.

3 | RESULTS

3.1 | Descriptive information

Descriptive information stratified by sex and AL is depicted in Tables 1 and 2. Women were significantly younger than men and reported significantly less emotional neglect, but a higher number of parental separation than men (Table 1). Subjects with no AL did not significantly differ from subjects with at least one AL with regard to the control variables (see 2.2.2), age or sex (all $p > 0.05$). AL only in childhood (<11 years old) was reported by 11 subjects. BDI scores were significantly positively correlated with anxiety (STAI-T; $r = 0.41$, $p < 0.001$), CTS Sum scores ($r = 0.15$, $p = 0.032$), and emotional neglect ($r = 0.14$, $p = 0.045$). STAI-T scores were significantly positively associated with CTS Sum scores ($r = 0.27$, $p < 0.001$) and emotional neglect ($r = 0.28$, $p < 0.001$). No significant associations of subcortical volumes with anxiety, depressive symptoms, CTS scores, or childhood attachment security were found (all $p > 0.06$).

3.2 | AI and amygdalar volumes

3.2.1 | No AL versus at least one AL

We investigated whether brain volumes differed between subjects that had reported at least one AL versus no AL during their entire life, and we observed that the experience of at least one AL versus no AL is associated with significantly larger bilateral amygdalar volumes (right amygdala: $\beta \pm SE = 0.049 \pm 0.019$, $p = 0.012$, p(FDR) = 0.036, partial $\eta^2 = 0.033$; left amygdala: $\beta \pm SE = 0.058 \pm 0.021$, $p = 0.005$, p(FDR) = 0.030, partial $\eta^2 = 0.040$) (Table 3, Figure 1). The association of AL with right and left amygdalar volumes stayed significant after correction for multiple comparisons and in all control analyses. Interestingly, in the control analyses, insecure childhood attachment and higher emotional neglect were also significantly associated with larger right amygdalar volumes (childhood attachment security: $\beta \pm SE = 0.037 \pm 0.016$, $p = 0.026$; CTS emotional neglect: $\beta \pm SE = 0.028 \pm 0.011$, $p = 0.016$).

The difference between no AL and at least one AL was significant for left amygdalar volumes irrespective of the time of AL occurrence [childhood/adolescence ($\beta \pm SE = 0.059 \pm 0.027$, $p = 0.029$), adulthood ($\beta \pm SE = 0.050 \pm 0.024$, $p = 0.037$), or both life periods ($\beta \pm SE = 0.061 \pm 0.025$, $p = 0.017$)]. For right amygdalar volumes, a similar pattern was found [childhood/

TABLE 1 Sample descriptives

	Whole sample			Δsex			ΔAL
	N = 196	Men	Women	*p*	AL = 0	AL > 0	*p*
AL (AL = 0/AL > 0)	42/154	26/72	16/82	0.082	–	–	–
Sex (m/f)	98/98	–	–	–	26/16	72/82	0.082
Total number of AL (*N* = 188)	1.76 ± 1.55	1.56 ± 1.51	1.95 ± 1.58	0.091	0	2.26 ± 1.40	–
Years since the last AL (*N* = 148)	4.5 ± 4.8	5.1 ± 4.9	4.0 ± 4.6	0.128	–	4.5 ± 4.8	–
Childhood attachment (secure/insecure)	105/91	53/45	52/46	0.886	20/22	85/69	0.383
Age (mean ± *SD*)	24.0 ± 3.2	24.6 ± 3.6	23.46 ± 2.59	0.011	23.2 ± 2.5	24.3 ± 3.34	0.059
BDI (mean ± *SD*)	2.29 ± 2.31	2.33 ± 2.24	2.26 ± 2.38	0.829	1.95 ± 2.26	2.38 ± 2.32	0.285
STAI-T (mean ± *SD*)	1.75 ± 0.40	1.75 ± 0.34	1.75 ± 0.45	0.943	1.72 ± 0.41	1.76 ± 0.40	0.629
CTS Sum (mean ± *SD*)	6.57 ± 2.06	6.58 ± 1.55	6.55 ± 2.48	0.918	6.69 ± 1.81	6.53 ± 2.13	0.661
CTS – emotional neglect (mean ± *SD*)	1.54 ± 0.73	1.67 ± 0.73	1.41 ± 0.72	0.011	1.71 ± 0.81	1.49 ± 0.71	0.083
Parental separation/ divorce in childhood (no/yes)	142/54	81/17	61/37	0.001	31/11	111/43	0.824

Note: Mean values, standard deviations (*SD*), or frequencies are listed for main and control variables, for the whole sample, for men and women, and for the groups with no AL and AL > 0 separately. *p* values of differences with regard to sex and AL in this sample are shown.

TABLE 2 Subcortical volumes

	Whole sample	Men	Women	Δsex	AL = 0	AL > 0
	N = 196	*N* = 98	*N* = 98	*p*	*N* = 42	*N* = 154
Right amygdala [cm^3]	0.87 ± 0.13	0.90 ± 0.13	0.83 ± 0.11	<0.001	0.83 ± 0.14	0.88 ± 0.12
Left amygdala [cm^3]	0.83 ± 0.13	0.87 ± 0.13	0.80 ± 0.11	<0.001	0.79 ± 0.14	0.85 ± 0.12
Right hippocampus [cm^3]	4.04 ± 0.37	4.17 ± 0.38	3.91 ± 0.31	<0.001	4.09 ± 0.31	4.02 ± 0.38
Left hippocampus [cm^3]	3.99 ± 0.37	4.13 ± 0.37	3.85 ± 0.32	<0.001	4.05 ± 0.36	3.98 ± 0.37
Right thalamus [cm^3]	6.31 ± 0.57	6.58 ± 0.57	6.03 ± 0.40	<0.001	6.36 ± 0.49	6.29 ± 0.59
Left thalamus [cm^3]	6.37 ± 0.57	6.63 ± 0.58	6.11 ± 0.43	<0.001	6.45 ± 0.52	6.35 ± 0.59
Right nucleus accumbens [cm^3]	0.34 ± 0.06	0.35 ± 0.06	0.33 ± 0.05	0.019	0.34 ± 0.04	0.34 ± 0.06
Left nucleus accumbens [cm^3]	0.39 ± 0.06	0.40 ± 0.06	0.38 ± 0.06	0.022	0.39 ± 0.05	0.39 ± 0.06
Right caudate [cm^3]	3.99 ± 0.46	4.11 ± 0.47	3.86 ± 0.41	<0.001	3.99 ± 0.46	3.99 ± 0.46
Left caudate [cm^3]	3.94 ± 0.45	4.07 ± 0.47	3.82 ± 0.40	<0.001	3.92 ± 0.45	3.95 ± 0.45
Right putamen [cm^3]	4.59 ± 0.50	4.82 ± 0.49	4.35 ± 0.37	<0.001	4.64 ± 0.53	4.57 ± 0.49
Left putamen [cm^3]	4.57 ± 0.50	4.79 ± 0.49	4.36 ± 0.40	<0.001	4.65 ± 0.53	4.55 ± 0.49
Total intracranial volume [cm^3]	1,471.5 ± 133.0	1,555.5 ± 114.6	1,387.5 ± 90.4	<0.001	1,476.7 ± 126.8	1,470.1 ± 135.0

Note: Mean values and standard deviations (*SD*) of the brain volumes are listed for the whole sample, for men and women, and for the groups with no AL and AL > 0 separately. *p* values of sex differences in this sample are shown.

adolescence ($\beta \pm SE = 0.048 \pm 0.025$, $p = 0.061$), adulthood ($\beta \pm SE = 0.048 \pm 0.022$, $p = 0.034$), or both ($\beta \pm SE = 0.054 \pm 0.024$, $p = 0.023$)], though weaker for AL in childhood/adolescence. The latter association was only significant after additionally controlling for the total number of AL ($p = 0.036$), for attachment-related measures ($p = 0.044$) or for emotional neglect ($p = 0.044$).

We did not observe sexually dimorphic associations between the dichotomous AL variable and amygdalar volumes in the main or control analyses (all $p \geq 0.08$, see Table SI-1).

3.2.2 | Exploratory analysis: Different types of AL

Weak, nonsignificant positive associations of amygdalar volumes with all three types of AL, namely the death of a first-degree relative or spouse, the death of a close friend or close relative, and the breakup of a steady relationship, were yielded in the main and control analyses (all $p > 0.18$, Table SI-5).

3.3 | AI and hippocampal volumes

3.3.1 | No AL versus at least one AL

Comparing subjects with at least one AL to those with no AL, no significant hippocampal volume differences emerged in the main analysis (Table 3, Figure 2), but after controlling for the total number of AL, right hippocampal volumes were significantly smaller in subjects with at least one AL ($\beta \pm SE = -0.137 \pm 0.063$, $p = 0.030$). Of note, the total number

TABLE 3 Main effect of AL (AL = 0/AL > 0) on subcortical volumes

	AL (AL = 0/AL > 0)	
	$\beta \pm SE$	*p* [*p*(FDR)]
R Amygdala [cm^3]	0.049 ± 0.019	0.012 [0.036]
L Amygdala [cm^3]	0.058 ± 0.021	0.005 [0.030]
R Hippocampus [cm^3]	−0.071 ± 0.051	0.168 [0.258]a
L Hippocampus [cm^3]	−0.071 ± 0.052	0.172 [0.258]
R Thalamus [cm^3]	−0.045 ± 0.067	0.499 [0.499]
L Thalamus [cm^3]	−0.076 ± 0.070	0.278 [0.334]
Exploratory analyses		
R Nucleus accumbens [cm^3]	0.007 ± 0.010	0.459
L Nucleus accumbens [cm^3]	−0.001 ± 0.010	0.892
R Caudate [cm^3]	0.013 ± 0.066	0.847
L Caudate [cm^3]	0.053 ± 0.065	0.416
R Putamen [cm^3]	−0.014 ± 0.066	0.829
L Putamen [cm^3]	−0.050 ± 0.071	0.458

Note: The β-estimates and standard errors (*SE*) of the multiple regression analyses, controlling for TIV, age, and sex, are listed for the association of AL with the investigated subcortical brain gray matter volumes. The *p* value after FDR correction for multiple comparisons is reported in parentheses in the right column.

[a]$p = 0.030$ after controlling for the total number of AL.

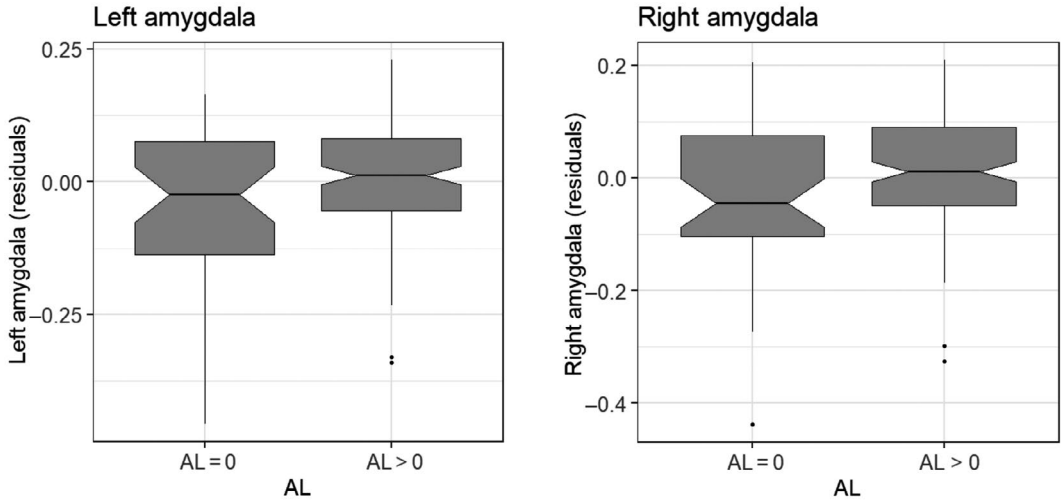

FIGURE 1 Main effect of affective loss (AL) on amygdalar volumes. The notched boxplots of the gray matter volumes of bilateral amygdalae (residuals, controlling for age, sex, and TIV) are depicted for AL (AL = 0/Al > 0). Significant differences were observed for bilateral amygdalar volumes that stayed significant in all control analyses

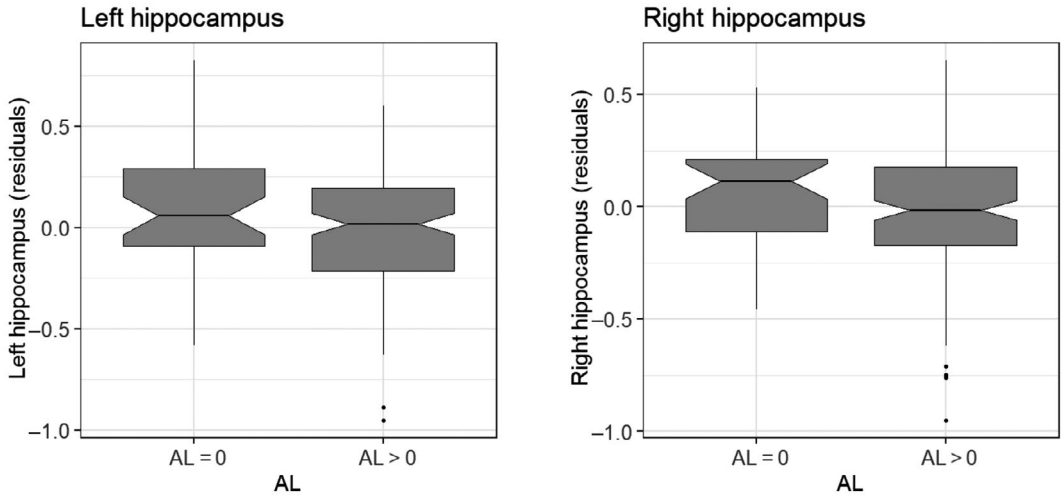

FIGURE 2 Main effect of affective loss (AL) on hippocampal volumes. The notched boxplots of the gray matter volumes of bilateral hippocampi (residuals, controlling for age, sex, and TIV) are depicted for AL (AL = 0/Al > 0). No significant differences were observed in the main analyses, but for right hippocampal volumes after additionally controlling for the total number of AL

of AL was weakly positively associated with right hippocampal volumes [$\beta \pm SE = 0.031 \pm 0.018$, $p = 0.076$, significant after controlling for clinical symptoms ($p = 0.036$)]. In addition, right hippocampal volumes were smaller, albeit nonsignificantly, in subjects who had reported AL only in either childhood/adolescence ($\beta \pm SE = -0.117 \pm 0.066$, $p = 0.077$), or adulthood ($\beta \pm SE = -0.104 \pm 0.058$, $p = 0.077$), but not in both life periods ($\beta \pm SE = 0.020 \pm 0.062$, $p = 0.742$). For left hippocampal volumes, no significant associations of AL > 0 versus AL = 0 in childhood/adolescence and/or adulthood were observed (all $p > 0.1$, see Table SI-2), but a weak negative linear association with the elapsed time after the last reported AL ($\beta \pm SE = -0.008 \pm 0.005$, $p = 0.098$, Table SI-4A) was found. We did not obtain significant sex differences for the association of the dichotomous AL variable with right or left hippocampal volumes in the main or control analyses (all $p > 0.33$).

3.3.2 | Exploratory analysis: Different types of AL

We did not detect significant associations between any type of AL and hippocampal volumes in the main analyses (all $p > 0.14$). However, after controlling for the total number of AL, right hippocampal volumes were significantly smaller in subjects who reported the loss of a first-degree relative/spouse ($\beta \pm SE = -0.198 \pm 0.082$, $p = 0.016$) or the loss of a close friend or relative ($\beta \pm SE = -0.150 \pm 0.061$, $p = 0.015$), but not after a relationship breakup ($\beta \pm SE = -0.050 \pm 0.055$, $p = 0.359$). Left hippocampal volumes exhibited a similar, but weaker pattern: After the loss of a close friend or relative, left hippocampal volumes were significantly smaller ($\beta \pm SE = -0.133 \pm 0.062$, $p = 0.033$), and after the loss of a first-degree relative/spouse nonsignificantly smaller ($\beta \pm SE = -0.162 \pm 0.083$, $p = 0.053$), and no differences were detected after a relationship breakup ($\beta \pm SE = 0.005 \pm 0.056$, $p = 0.924$).

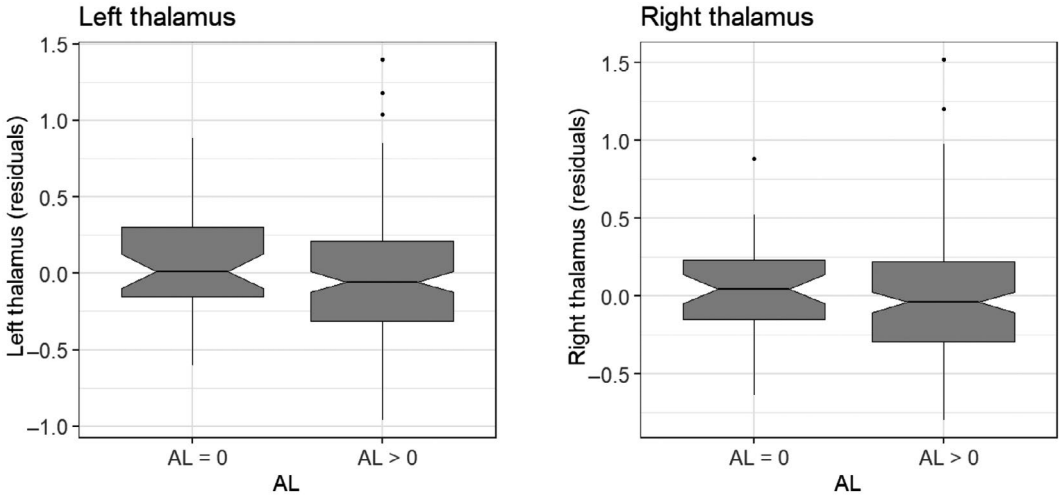

FIGURE 3 Main effect of affective loss (AL) on thalamic volumes. The notched boxplots of the gray matter volumes of bilateral thalami (residuals, controlling for age, sex, and TIV) are depicted for AL (AL = 0/Al > 0). No significant differences were found for the thalami in the ROI analyses

3.4 | Al and thalamic volumes

3.4.1 | No AL versus at least one AL

Thalamic volumes were smaller after at least one AL compared to no AL, but the difference was nonsignificant in the main (all $p > 0.27$) and in the control analyses (all $p \geq 0.19$) (Table 3, Figure 3). Similarly, AL > 0 versus AL = 0 in childhood/adolescence and/or adulthood was nonsignificantly associated with thalamic volumes (Table SI-2). There was no evidence for sexually dimorphic associations (all $p > 0.26$). However, the exploratory whole-brain analysis revealed smaller right temporal, visual and parietal thalamic volumes in AL > 0 compared to AL = 0 at an uncorrected significance threshold ($p < 0.001$, Table SI-6, Figure SI-1), and this finding was accompanied by larger gray matter volumes of left middle occipital gyrus and right inferior temporal gyrus ($p < 0.001$, Table SI-6) in AL > 0 compared to no AL.

3.4.2 | Exploratory analysis: Different types of AL

Different types of AL were not significantly related to thalamic volumes, neither in the main nor in the control analyses (all $p > 0.19$) (Table SI-5).

3.5 | Exploratory analyses: AL and striatal volumes

3.5.1 | No AL versus at least one AL

Exploring left and right nuclei accumbens, caudate and putamina volumes, no significant striatal volume differences between AL = 0 and AL > 0 were observed in the main or control analyses (Table 3). The analyses of AL > 0 versus AL = 0 in childhood/adolescence and/or adulthood did not yield significant results either (Table SI-2). However, a significant sexually dimorphic relationship between the dichotomous AL variable and right nucleus accumbens volumes was found that stayed significant in all control analyses (AL × sex interaction: $\beta \pm SE = 0.041 \pm 0.019$, $p = 0.035$, Table SI-1, Figure 4). Men with at least one AL compared to no AL displayed larger nucleus accumbens volumes than women. Post hoc multiple regression analyses, performed in men and women separately, revealed that AL-related differences were significant in men ($\beta \pm SE = 0.026 \pm 0.013$, $p = 0.0497$), but not in women ($\beta \pm SE = -0.018 \pm 0.014$, $p = 0.208$). No further significant sexually dimorphic effects of AL on striatal volumes were found. However, the total number of AL was positively associated with right nucleus accumbens volumes after additionally controlling for clinical symptoms ($\beta \pm SE = 0.01 \pm 0.00$, $p = 0.036$), and this positive relation was observed both in men ($p = 0.066$) and, though less pronounced, in women ($p = 0.371$).

3.5.2 | Different types of AL

The loss of a first-degree relative/spouse was associated with larger left caudate volumes ($\beta \pm SE = 0.196 \pm 0.094$, $p = 0.038$). This association stayed significant in all control analyses except after controlling for the total number of AL ($p = 0.129$). No other significant results were observed in the main analyses (all $p > 0.06$) (Table SI-5). After controlling for childhood maltreatment (CTS Sum), larger right caudate volumes were associated with the loss of a first-degree relative/spouse ($\beta \pm SE = 0.206 \pm 0.097$, $p = 0.036$).

4 | DISCUSSION

With this study, we investigated the association between AL experiences and subcortical gray matter volumes. To the best of our

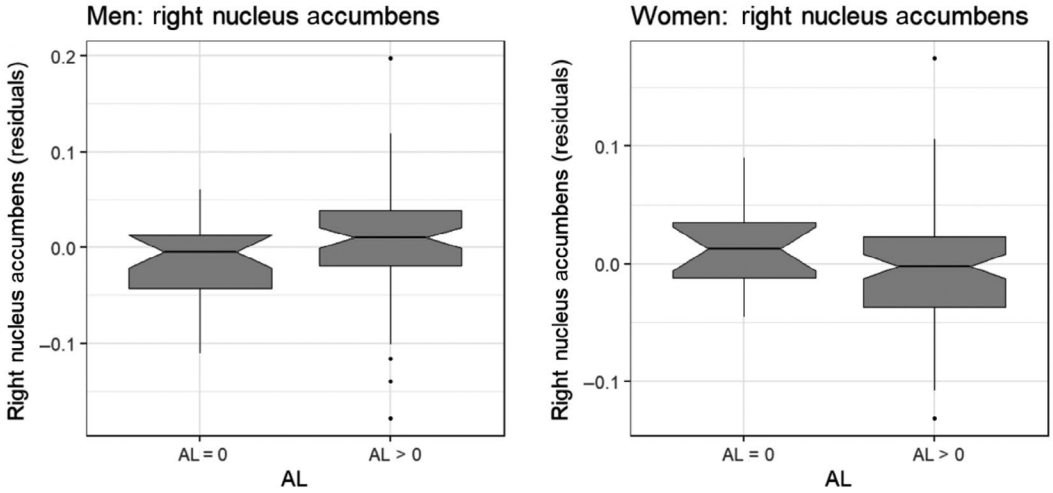

FIGURE 4 Sex-specific effect of affective loss (AL) on right nucleus accumbens volumes. The notched boxplots of the gray matter volumes of right nucleus accumbens (residuals, controlling for age and TIV) are depicted for the interaction of AL (AL = 0/Al > 0) with sex. *Post hoc* analyses showed that the dichotomous AL variable was significantly associated with nucleus accumbens volumes in men, but not in women

knowledge, our study is the first to highlight the impact of AL on amygdalar volumes in healthy young adults. We found that the experience of at least one AL compared to no AL is associated with larger bilateral amygdalar volumes. Amygdalar volume alterations were observed irrespective of whether AL occurred only in childhood/adolescence, only in adulthood or in both life periods. In addition, amygdalar volume increases were linked to all three investigated types of AL, albeit nonsignificantly so.

Further, right hippocampal volumes were smaller in subjects with at least one AL compared to no AL. However, this relationship was only significant after controlling for the total number of AL, which showed a weak positive association with right hippocampal volumes in the subgroup of persons with AL.

No AL-related alterations in thalamic volumes were found in the region-of-interest analyses, but the whole-brain analysis yielded a weak modulation of temporal, visual, and parietal thalamic volumes by AL, showing smaller thalamic volumes in subjects with at least one AL compared to those with no AL.

In exploratory analyses of striatal volumes, a sexually dimorphic association between AL and nucleus accumbens volumes was discovered, showing that the experience of AL was related to larger right nucleus accumbens volumes in men compared to women. Furthermore, the loss of a first-degree relative was associated with larger left caudate volumes.

Amygdala and hippocampus are part of the limbic network and play key roles in stress physiology, as well as in psychiatric disorders such as GAD and MDD, among others. Our findings are in accordance with a large body of animal studies showing amygdalar hypertrophy and hippocampal atrophy after chronic stress exposure (e.g., Magariños & McEwen, 1995; Magariños et al., 1996; Vyas et al., 2002).

The amygdala plays a key role in emotion processing and stress physiology, stimulating autonomic and neuroendocrine stress responses and enhancing fear learning via thalamic and hippocampal pathways (Insausti & Amaral, 2012; Kim et al., 2015; Tully et al., 2007; Ulrich-Lai & Herman, 2009; Yilmazer-Hanke, 2012). Larger amygdalar volumes have been related to heightened anxiety in animal and human studies (De Bellis et al., 2000b; Etkin et al., 2009; Schienle et al., 2011; Vyas et al., 2002), to increased stress excitability and to decreased reward in animals (Ulrich-Lai & Herman, 2009). Larger amygdalar volumes have also been observed in association with chronic occupational stress (Savic, 2015), emotional neglect in childhood (Mehta et al., 2009; Roth et al., 2018; Tottenham et al., 2010), an association supported by our study results after controlling for AL, and with first-episode depression (Frodl et al., 2002, 2003). In animal studies, repeated social defeat and chronic immobilization stress increased dendritic arborization in the basolateral amygdala (BLA) of male rats (Patel et al., 2018), and acute and chronic immobilization stress resulted in a higher BLA spine density paralleled by higher behavioral anxiety (Mitra et al., 2005; Vyas et al., 2002). In summary, our results showed that AL is associated with amygdalar volume alterations typically observed after chronic stress exposure, and comparable with findings in GAD, first-episode depression and emotional neglect in childhood. Interestingly, our data also showed that larger right amygdalar volumes are linked to insecure childhood attachment.

In animal studies, the amygdalar hypertrophy after chronic stress persisted even after a stress-free recovery period (Vyas et al., 2004). Comparably, our study results suggested that amygdalar alterations do not decrease over time after the AL experience, and persist from childhood on. This might explain why studies, assessing stressful life events only during a limited time period and not in whole life, did not report enlarged amygdalar volumes in association with stressful life events (Benetti et al., 2010; Gianaros et al., 2007; Papagni et al., 2011).

The amygdala enhances fear learning via the hippocampus, but even more so appears to be crucial for conveying effects of stress

on the hippocampus (Kim et al., 2015). In rats, inactivation or lesion of the amygdala blocked stress effects on hippocampal long-term potentiation (LTP) irrespective of glucocorticoid levels, and electrical stimulation of the amygdala suppressed hippocampal CA1 LTP (Kim et al., 2015). However, the connection between neurophysiological changes in the amygdala with those in the hippocampus is not yet well understood (Kim et al., 2015). The hippocampus, abundant in corticosteroid receptors, is crucially involved in memory and inhibitory feedback regulation of the HPA axis (Insausti & Amaral, 2012; Ulrich-Lai & Herman, 2009). Chronic stress in rats induced dendritic atrophy and debranching in hippocampal CA3 pyramidal neurons (Vyas et al., 2004), and dendritic atrophy in the hippocampal CA1 (Patel et al., 2018). While chronic stress selectively reduced overall hippocampal volumes (Lee et al., 2009), Pinto et al. (2015) showed that volumetric reductions following chronic stress might be restricted to the dorsal hippocampus, predominantly implicated in cognitive functions, while the volume of the ventral hippocampus, involved in behavioral inhibition and emotional memory, might even increase (Pinto et al., 2015; Segal et al., 2010). Correspondingly, chronic stress resulted in an elevated activity of the ventral compared to the dorsal hippocampus (Pinto et al., 2015) dovetailing with behavioral observations that chronic stress is related to impaired hippocampus-dependent performance in declarative memory and spatial navigation tasks in animals and humans, as well as to decreased negative feedback regulation of stress (Kim et al., 2015; Ulrich-Lai & Herman, 2009; Vyas et al., 2002), but to enhanced amygdala- and hippocampus-dependent contextual fear conditioning (Kim et al., 2015). Similarly, in a human neuroimaging study, comparing subjects with GAD to healthy controls, smaller hippocampal volumes correlated with lower functional hippocampal activation during an explicit verbal memory task and with a worse recognition performance (Moon et al., 2015). Hippocampal volume reductions have been consistently linked to GAD (Hettema et al., 2012; Moon et al., 2014, 2015), to MDD (Arnone et al., 2012; McKinnon et al., 2009), early life adversity (Rao et al., 2010), childhood maltreatment (Calem et al., 2017; Chaney et al., 2014), post-traumatic stress disorder (Bremner, 1999; Karl et al., 2006), the number of stressful life events in the past 3 months (Papagni et al., 2011), and perceived stress during the past 20 years (Gianaros et al., 2007). We conclude that AL is associated with hippocampal volume alterations usually induced by acute and chronic stress, and consistently found in individuals with GAD, MDD, and childhood maltreatment.

Interestingly, the total number of AL proved to be relevant for right hippocampal volumes alterations in our study, showing a positive correlation with these volumes in our subgroup with at least one AL. We speculate that a higher number of AL is not only an indicator of a more intense and chronic stress exposure, but might also give rise to fear conditioning that has been associated with the ventral hippocampus (Kim et al., 2015; Pinto et al., 2015). Thus, a higher number of AL might be related to stronger ventral hippocampal volume increases as a consequence of fear conditioning, mitigating stress-related dorsal hippocampal volume reductions.

Our analyses further suggest that bereavement, that is, the loss of a first-degree relative or close friend/relative, rather than the breakup of a relationship, is related to hippocampal atrophy. Of note is that our subjects were all students whose relationships were limited in duration given their young age. The breakup of a longer lasting relationship later in life might lead to different results.

In rodents, stress-related hippocampal atrophy was reversible after a stress-free recovery period (Conrad et al., 1999). However, our data did not provide evidence for a reversibility of bilateral hippocampal volume reductions over time. By contrast, our data hinted at a—even though very weak—linear decrease of left hippocampal volumes over time after the experience of AL. Hence, our data rather support the notion of a long-lasting hippocampal stress response after AL which might even increase over time.

AL was not associated with significant alterations in thalamic volumes in our region-of-interest analyses. In previous neuroimaging studies, grieving for an AL has modulated left thalamic activations (Kersting et al., 2009; Najib et al., 2004). The thalamus, in particular the paraventricular nucleus, has been shown to convey the effects of chronic stress on autonomic excitability (Ulrich-Lai & Herman, 2009). Smaller thalamic volumes have been reported in individuals with GAD and MDD (e.g., Moon et al., 2014; Nugent et al., 2013). The thalamus is a complex structure comprising several collections of nuclei, and it is conceivable that the investigation of thalamic substructures, such as the paraventricular nucleus volume, might be a more sensitive measure of stress-related effects. In support of this view, our whole-brain analysis revealed smaller volumes in the right temporal, visual and parietal thalamus in subjects with at least one AL compared to those with no AL. In the same analysis, AL > 0 was weakly associated with larger occipital and inferior temporal gyrus volumes compared to AL = 0. Hence, our data suggest a weak modulation of visual processing by AL in thalamic, occipital, and temporal regions, which might also involve the amygdala. The amygdala has been shown to enhance sensory processing of affective significant stimuli via the visual cortex (Lim et al., 2009; Pessoa & Adolphs, 2010). Of note, our whole-brain analysis did not yield gray matter volume differences in the amygdala. Another study has already shown that VBM-based results are not necessarily comparable to segmentation results (Giuliani et al., 2005), due to differences in the methodological approach. In addition, the identification of the amygdala might be less accurate compared to other subcortical structures in VBM (Focke et al., 2014).

While our exploratory analyses of striatal volumes did not yield main effects of AL, larger nucleus accumbens volumes were observed in men, but not in women, comparing subjects with at least one AL to no AL The nucleus accumbens is essential for approach motivation during reward processing, implicated in responsiveness to rewards (Novick et al., 2018) and involved in passive stress coping after separation from the female partner in monogamous male mammals (Bosch et al., 2008, 2016). To the best of our knowledge, little is known about sex-specific stress effects on the nucleus accumbens, as most studies investigated only one sex. Sex-specific differences

are conceivable given the high density of sex steroid receptors in the nucleus accumbens (Goldstein et al., 2001): In male mammals, stress exposure and stress-induced anhedonia have been linked to increased spine density, dendritic hypertrophy, and larger volumes of the nucleus accumbens (Bessa et al., 2013; Warren et al., 2014). In human adolescents of both sexes, larger nucleus accumbens volumes have been related to peer problems, but interactions with sex have not been probed (Lee et al., 2020). In a neuroimaging study with women, bilateral ventral striatal activations have been decreased by grieving for an AL (Najib et al., 2004). Our findings in men are in line with studies reporting enlarged nucleus accumbens volumes after stress exposure in male animals and adolescents of both sexes (Bessa et al., 2013; Lee et al., 2020; Warren et al., 2014). Increased nucleus accumbens volumes have also been linked to anxiety or depression (Günther et al., 2018; Kühn et al., 2011; Lee et al., 2020). However, it remains elusive why the association of nucleus accumbens volumes with AL was weaker in women in our study.

Interestingly, our exploratory analyses also indicated a positive relationship between caudate volumes and the loss of a first-degree relative. The caudate is implicated in reinforcement-based learning, the flexible adaptation of behavior to internal and external cues and hence crucial for goal-directed behavior (Graybiel & Grafton, 2015; Haber et al., 2012). Parts of the caudate nucleus might even be causally involved in the generation of repetitive choices based on negative evaluations (Amemori et al., 2018). Studies in adults showed that caudate volumes are reduced in MDD (Arnone et al., 2012; Bora et al., 2012; Krishnan et al., 1992) as well as in childhood maltreatment (Frodl et al., 2017), but enlarged in GAD (Hilbert et al., 2015). Reduced caudate volumes correlated with higher anhedonia and overall depressive symptoms in adults (Pizzagalli et al., 2009), and larger striatal volumes including the caudate have been associated with more severe worries and a higher intolerance of uncertainty (Hilbert et al., 2015). Hence, the loss of a first-degree relative seems to be associated with caudate volume alterations as observed in GAD, but not in MDD or childhood maltreatment, and might be indicative of a state of worries and higher intolerance of uncertainty. In sum, our data support the view that AL versus no AL is linked to striatal volume alterations indicating effects of AL on reward processing.

Taken together, our data provide evidence that AL experiences are associated with subcortical structure alterations and might thus render an individual more vulnerable to future stress exposure and to the development of psychiatric diseases such as an anxiety or MDD. Men compared to women might exhibit an even enhanced vulnerability as the sex-specific association with nucleus accumbens volumes suggests. Our findings highlight the importance of stable human attachments for stress resilience and mental health in humans. In our study, an insecure attachment in childhood was linked to similar brain structural changes in the right amygdala. Thus, we speculate that AL experiences—beyond constituting a stressful experience per se—might also shake the attachment security of individuals. Future studies could address whether the access to a strong social support network or therapeutic interventions targeting attachment security mitigate the associations of AL experiences with subcortical brain structures in the afflicted individual.

4.1 | Limitations

The number of ALs was assessed retrospectively, and our sample reported a rather low number thereof. However, we investigated a representative sample of students. We included only students in our study in order to achieve a homogeneous sample: While this strategy reduces potential confounder effects, it might also limit the generalizability of our study results.

We did not assess menstrual cycle phase or the use of oral contraceptives in women. There is emerging evidence that the menstrual cycle or the use of contraceptives affect female brain structure, and modulate subcortical structures including the hippocampus (Catenaccio et al., 2016; Lisofsky et al., 2015; Pletzer et al., 2010; Protopopescu et al., 2008). Moreover, we did not assess the coping strategies that participants applied to deal with the AL experience.

It would be worthwhile to replicate and extend our results in a longitudinal study which would allow to assess AL experiences and associated brain structural changes prospectively.

4.2 | Conclusions

AL was associated with larger bilateral amygdalar volumes irrespective of sex, with larger nucleus accumbens volumes in men, and, after controlling for the total number of ALs, with smaller right hippocampal volumes in both sexes. We found evidence that the loss of a first-degree relative was associated with larger caudate and smaller hippocampal volumes. Our data suggest that AL engenders alterations in limbic structures that likely involve processes of chronic stress and amygdala- and hippocampus-dependent fear conditioning, and that were comparable to those observed after childhood maltreatment, in MDD and general anxiety disorder. Our exploratory findings of striatal volume alterations further suggest that AL exerts effects on reward processing.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

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INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, T.K. and H.A.; *Methodology*, H.A., A.J., and T.K.; *Investigation*, H.A. and A.J.; *Formal Analysis*, H.A.; *Data Curation*, H.A.; *Project Administration*, T.K. and H.A.; *Writing – Original Draft*, H.A.; *Writing – Review & Editing*, T.K. and A.J.; *Visualization*, H.A.; *Funding Acquisition*, T.K., A.J.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jnr.24835.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

TABLE SI-1 Interaction of AL (AL = 0/AL > 0) and sex on subcortical volumes

TABLE SI-2 Main effects of AL in childhood/adolescence and/or in adulthood on subcortical volumes

TABLE SI-3 Association of the total number of AL with subcortical volumes

TABLE SI-4A Linear association between the time that has elapsed since the last AL and subcortical volumes

TABLE SI-4B *U*-shaped association between the time that has elapsed since the last AL and subcortical volumes

TABLE SI-5 Association between different types of AL and subcortical volumes

TABLE SI-6 Whole-brain analysis comparing AL = 0 with AL > 0

FIGURE SI-1 Contrast [(AL = 0) > (AL > 0)]

Transparent Science Questionnaire for Authors

Transparent Peer Review Report

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