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onset. In keeping with the view of adolescence as a time of particular vulnerability to developmental perturbations, cross-sectional studies reveal that regionally speci c e ects of early stressors in the amygdala are most prominent during adolescence [18]. We therefore examined currently healthy young people who self-reported early exposure to a range of extreme stressors. Self-report measures of early trauma (natural disasters, family illness/death, experiences of bullying etc.) have been previously linked to reduced amygdala volumes in young people [18,26,27], however the impact of such stressors on functional connectivity in youth have not been widely explored [28]. Concurrent examinations of trauma-related changes in both brain structure and functional connectivity have been limited to adult populations or to task-speci c activation paradigms [16,29].

e current study employed structural and functional magnetic resonance imaging to examine grey and white matter volume estimates by means of voxel based morphometry (VBM), as well as e ective functional connectivity by means of spectral dynamic causal modelling (spectral DCM; spDCM) within the amygdala-hippocampal network in currently-healthy adolescents. Importantly, any neural di erences between stress exposed individuals versus unexposed individuals should not be driven by current psychopathology, as all participants were free from current psychopathology.

## Materials and Methods

### Participants and centers

A large cohort of 298 healthy adolescents [152 males, range: 14-24 years, mean = 19.1 ± 2.9 (SD); 146 females, range: 14-24 years, mean = 19.1 ± 2.9] were scanned over 1½ years at 3 sites: (1) Wellcome Trust Centre for Neuroimaging (WTCN), London, (2) Medical Research Council Cognition and Brain Sciences Unit (MRC CBSU), Cambridge, and (3) Wolfson Brain Imaging Centre (WBIC), Cambridge. e study received ethical approval from the NRES Committee East of England - Cambridge Central (12/EE/0250) and all participants gave written informed consent. is study was conducted by the NeuroScience in Psychiatry Network (NSPN), which addresses how psychiatric disorders are related to abnormal maturation of brain systems.

### Selection of participants with histories of abuse and neglect

e Structured Clinical Interview for DSM-IV (SCID) was administered by a trained research assistant and audio recordings were made with the informed consent of participants. During the course of these interviews 29 participants were identi ed (10 female, mean age 20.45 years) who self-reported histories of trauma (a solitary trauma), including experiences of physical or sexual abuse, having been in a life-threatening situation (e.g. natural disaster, car accident, drowning), physical/sexual assault, death of a parent or sibling or witnessing/ hearing about actual or threatened death to others over the course of childhood. e control group (mean age: 20.53 years) was created by matching each trauma-exposed participant to a non-exposed participant in terms of gender, age and parental education levels (as a proxy for socio-economic status). ere was no signi cant di erence in handedness between the two groups. Table 1 shows demographic data of participants.

| | Trauma-exposed participants (N = 29) | Non-exposed participants (N = 29) | p-values |
|---|---|---|---|
| Age [years] | 20.53 ± 2.77 range 16-25 | 20.45 ± 2.61 range 16-25 | 0.9087 |
| Sex [Male/Female] | 19/10 | 19/10 | 1.0000 |
| Parental education [years] | 18.05 ± 8.03 | 16.15 ± 6.18 | 0.3153 |

Table 1: Demographic data of selected participants.

### Data acquisition and preprocessing

#### Structural MRI

All multi-parameter maps (MPM) were acquired on 3T whole body MRI systems (Magnetom TIM Trio, Siemens Healthcare, Erlangen, Germany; VB17 so ware version) operated with the standard 32-channel radio-frequency (RF) receive head coil and RF body coil for transmission. e MPM comprised three multi-echo 3D fast low angle shot (FLASH) scans with PD (TR/α = 23.7 ms/60), T1 (TR/α = 18.7 ms/$20^0$), and MT (TR/α = 23.7 ms/$6^0$) - weighted contrast, one RF transmit (B1) eld map and one static magnetic (B0) eld map scan [30].

e MPM acquisition and pre-processing were developed and optimized in previous studies and are widely described elsewhere [30-36]. e post-processed MT maps resulting from this step were used in our VBM analyses.

#### Functional MRI

At all three sites, fMRI data were acquired on 3T whole body MRI systems (Magnetom TIM Trio, Siemens Healthcare, Erlangen, Germany; VB17 so ware version) operated with the standard 32-channel radio-frequency (RF) receive head coil and RF body coil for transmission. 269 contiguous multi-slice images were obtained with a multi-echo-planar sequence (orientation = AC-PC line, number of slices = 34; slice thickness = 3.8 mm; FOV = 240 mm; TE1 = 13 ms; TE2 = 31 ms; TE3 = 48 ms; TR = 2.420 s; ip angle = 90°; matrix size = 64×64×34; voxel size = 3.8×3.8×3.8 $mm^3$).

e fMRI data were analysed using procedures implemented in Statistical Parametric Mapping (SPM8, Welcome Trust Centre for Neuroimaging, London, UK; http://www. l.ion.ucl.ac.uk/spm). First, the fMRI data were summed up over the three echoes. Data were then realigned, co-registered, anatomical images were normalized to MNI space, and the resultant normalization matrix was then used to normalize the fMRI data. Finally, the data were visually inspected and spatially smoothed using a 6 mm Gaussian kernel. Ultra-low frequency uctuations were removed using a high-pass lter (1/128 s, 0.0078 Hz). Confound time-series were extracted from prede ned coordinates of extra-cerebral compartments (the pons: x, y, z = 0, -24, -33; and lateral ventricle: x, y, z = 1, -43, 6).

We extracted data exhibiting physiologically-relevant resting-state (i.e. low frequency) dynamics from our region(s) of interest (ROIs): le and right amygdala, and le and right hippocampus, which were anatomically de ned using the PickAtlas so ware (WFU PickAtlas, ANSIR Laboratory, Winston-Salem, NC, USA; http://fmri.wfubmc.edu/so ware/PickAtlas). e resting-state was thus modelled using a General Linear Model (GLM) with a discrete cosine basis set (GLM-DCT) consisting of 130 functions with frequencies characteristic of resting-state dynamics (0.0078 – 0.1 Hz [37-40]), six nuisance regressors capturing head motion, and the confound time-series from the extra-cerebral compartments. e regional BOLD signal was summarized with the principal eigenvariate (adjusted for confounds: head movements and extra-cerebral compartments) of voxels within 6 mm of the subject's peak coordinate, as identi ed using statistical parametric mapping. For those familiar with the process of extracting ROIs, this was achieved by using an F-contrast including the discrete

cosine set modelling the resting-state. is procedure allowed us to extract physiologically relevant resting-state data from the anatomically de ned regions for each hemisphere.

**Spectral dynamic causal modelling (spDCM). E ective connectivity estimates.** Spectral dynamic causal modelling (spectral DCM; spDCM) is based on deterministic models that generate predicted crossed spectra from a biophysically plausible model of coupled neuronal uctuations in a distributed neuronal network [41]. In this setting, the nature of the endogenous uctuations (and ob9(io)10.9(36)12(uc)-29 TD.u 1I10(u)-...u 1saRM)(h)3(a)3(s )0.5 tobps a.9(ra9[(m)4e(t)6((r)-6(zce)-5(d)6(. )0.5(h)3.9(e)10.1( rom(o)12erere24(-1
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## Multiple linear regression models

To test the hypotheses that spDCM parameter estimates were di erent between groups, we carried out linear regression analyses.

We rst calculated the averaged spDCM parameters from resting-state fMRI data. is was followed by a multiple regression analysis explaining spDCM estimates by a constant, age, di erentiating between males and females (in order to identify gender-speci c e ects), poverty scores, group, and scanner site. e correlation was modelled as:

$spDCM_i = \beta_1$

| | $GMV_i$ = f(group)<br>Negative associations<br>p-values (unc.) |
|---|---|
| GMV (rAMYG) | 0.3627 |
| GMV (rHF) | 0.2938 |
| GMV (lAMYG) | 0.4837 |
| GMV (lHF) | 0.1808 |

Table 3: Significance levels (p-values) for negative associations between group

the spectral DCM algorithm together with the Bayesian comparison procedure implemented here may be further improved by searching for optimal region-speci c uctuation models (and observation noise) rather than assuming the same generative model across regions (from AR1 up to AR16 processes). Although that approach may have advantages and potentially reveal some other statistical e ects, it is computationally very expensive due to estimation of a very large number of models. Second, analyses suggest the use of other statistical correction strategies rather than FDR (e.g. a random eld theory approach). e reason for this is that FDR does not take into account autoregressive models (i.e. the temporal structure), nor brain regions (i.e. the spatial structure) and deals with all parameter estimates as independent of each other. ird, VBM is commonly directed at examining gray matter but it can also be used to examine white matter. In the latter case, however, the sensitivity is limited because white matter areas are characterized by large homogeneous regions with only subtle changes in intensity [51]. Finally, the use of the SCID interview to select participants with traumatic life events may be criticized as being too structured (and diagnostically focused), potentially not enabling all former traumatic experiences to be revealed.

In summary, our ndings demonstrated abnormal structural-functional maturation of the right amygdala in currently-healthy young people exposed to traumatic events. Together, these ndings are suggestive of potential biological markers over the course of adolescence that may have prognostic utility for PTSD or depression. Indeed, our observations, both in white-matter and intrinsic connectivity within right amygdala are very interesting and intriguing, however the reason, i.e. the underlying biological mechanisms that lead to these observations, remain an open question since only a small body of research has been conducted on this topic so far.

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