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The RAISE study protocol; a cross-sectional, multi-level, neurobiological study of studying resilience after individual stress exposure

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ABSTRACT

Introduction: This paper describes the protocol for an ongoing project funded by the Royal Society, the Resilience After Individual Stress Exposure (RAISE) study; which aims to examine the factors and mechanisms that facilitate resilient functioning after childhood adversity (CA). **Methods and Analysis:** We aim to recruit up to 200 participants. We will use dimension reduction techniques (PCA) on standard-normally transformed individual parameters of mental health, social functioning and CA to calculate a composite measure of adaptive (i.e., 'resilient') psychosocial functioning. To examine the neuro-immune responses to stress and their relationship with the brain and social environment, we will use a well validated functional magnetic resonance imaging (fMRI) task; the Montreal imaging stress task (MIST), and venepuncture. We will run group or dimensional comparisons in multiple levels of biological and psychological outcomes, as well as mediation and moderation analyses to study how key biological systems (i.e., the hypothalamic-pituitary-adrenal axis and the immune system) interrelate and interact with brain function and social influences in order to facilitate resilient functioning after CA. We hypothesize that resilient functioning will be facilitated by reduced morning cortisol and cytokine levels before and after the stressor and improved neural responses to such stress, as well as increased gray matter volume in the hippocampus and prefrontal cortex, enhanced inhibitory control and emotion regulation, and more friendship and family support. **Ethics and Dissemination:** This study has been reviewed and given favourable opinion by the National Research Ethics Service, NRES Committee East of England – Cambridge Central and external reviewers from the Royal Society (RGF\R1\180064 and RGF\EA\180029). The results of the RAISE study will be disseminated through (1) publications in scientific peer reviewed journals, (2) presentations on relevant scientific conferences and meetings, (3) publications and presentations for the general public and (4) through social media.

Keywords: Resilience; Stress; Childhood adversity; Maltreatment; Adolescence.

Strengths and limitations of this study

The RAISE study will provide a comprehensive evaluation of the neurobiological mechanisms that contribute to adolescent resilience.

The study assesses the mechanisms that contribute to adolescent resilience incorporating standardised and validated instruments of psychological functioning, childhood adversity, cognitive tasks, venepuncture and neuroimaging.

The findings will help inform intervention strategies for individuals who have experienced childhood adversity in order to prevent the development of mental health disorders, and ultimately increase resilience.

Child adversity will be assessed from self-reports subjected to reporting biases.

A longer recruitment period may be required due to Covid-19.

1. INTRODUCTION

Up to a third of children worldwide experience childhood adversity (CA) within the family environment (1,2). CA within the family environment comprises childhood maltreatment (including emotional, sexual, and physical abuse, and emotional and physical neglect) and intra-family adversity (including marital distress/conflict, parental alcohol dependence, aggressive parenting behaviours, parental violence, parental mental health problems, and stressful family life events) before the age of 16 (3).

CA is the leading preventable risk factor for mental illness and substance abuse (4-11). It can have a detrimental impact on a wide range of functions. For example, CA has been associated with physical (e.g., failure to thrive, poor adult health, and high mortality), cognitive (e.g., impaired inhibitory control and emotion regulation), and personal and interpersonal problems (e.g., negative self-cognitions, suicidal behaviours, increased peer rejection, social withdrawal, sexual maladjustment, aggression, and criminality) (11-19).

Importantly, adolescents with CA are at increased risk for and are more sensitive to psychosocial stress. In response to acute stress, the body reacts by releasing pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) (20). These cytokines play a key role in stress reactivity and stress recovery (21). Specifically, pro-inflammatory cytokines stimulate the hypothalamic-pituitary-adrenal (HPA) axis to release glucocorticoid hormones such as cortisol. Glucocorticoids, in turn, suppress the further release of cytokines from the immune system (22). Thus, cortisol is an important anti-inflammatory compound in the body that is crucial for stress recovery. Pro-inflammatory cytokines and chemokines can cross the blood-brain barrier and negatively impact the function of brain regions involved in threat, reward, and executive functioning (23,24). Indeed, acute stress is associated with increased levels of pro-inflammatory cytokines in the amygdala and decreased pro-inflammatory cytokines in the medial prefrontal cortex (MPFC) (25); regions associated with executive functions and emotion regulation (26-31). Therefore, it is plausible that the alteration of these processes, or the inability to properly value and manage emotions, can lead to anxiety and/or depression in situations of negative affect (32-34).

However, although CA is associated with considerably lowered odds of adequate mental and physical health functioning later in life, a significant proportion of individuals with a history of CA function 'better than expected,' or, in other words, are "functioning resiliently" (35). Resilient functioning after CA is thought to be facilitated by protective 'resilience factors' that help individuals to adapt and recover from, or compensate for, the sequelae of CA (36, Figure 1). However, it is yet unknown whether and how neuro-immune responses to psychosocial stress differ in resilient vs. vulnerable adolescents with a history of CA.

2. OBJECTIVES AND HYPOTHESIS

Resilient functioning has been associated with various somatic components, ranging from genes and cellular mechanisms to higher-order biological systems and the social environment (see reviews in 36-38; Figure 1). In this study, we aim to test the factors and mechanisms that facilitate resilient functioning, the interactions between those factors, and how they explain resilient responses to future stress. Specifically, we will address resilience by investigating how key biological systems (i.e., the HPA axis and the immune system) interrelate and interact with brain structure, brain function, and social influences to facilitate resilient functioning after CA.

All included participants will complete an online assessment to assess psychological functioning and early life experiences, an in-unit assessment day to assess neuro-immune and cognitive responses to stress, and an online follow-up assessment to assess psychological functioning after stress exposure. Resilient functioning will be quantified as the degree to which an individual functions better or worse than expected given their self-reported childhood family experiences (35; Figure 2). To examine the neuro-immune responses to stress and their relationship with brain structure and function and the social environment, we will use a well-validated functional magnetic resonance imaging (fMRI) task, The Montreal imaging stress task (MIST), and venepuncture. Since stress increases circulating inflammatory protein levels in the blood, and high levels of inflammation predict later mental health disorders, we will examine whether resilience is related to lower levels of inflammation in response to psychosocial stress, and whether this is explained by improved brain responses to stress.

We hypothesise that resilient functioning will be facilitated by:

1) Reduced morning cortisol and cytokine levels before and after the stressor (i.e., a latent factor constructed by serum high-sensitivity c-reactive protein (hs-CRP), TNF-α, IL-6 and Interleukin-1 factor beta (IL1β) levels, as these have been shown to be increased in those with CA and mental illness (i.e., 39-43)).
2) Reduced stress-related brain responses (i.e., amygdala, insula) and increased modulatory responses in the MPFC and anterior cingulate cortex (ACC) (44-46) during the MIST.
3) Increased functional connectivity between the DMPFC and emotion processing regions (e.g., insula, amygdala and hippocampus) during the MIST (47).
4) Increased gray matter volume in the hippocampus and prefrontal cortex (48-54).
5) Enhanced cognitive and emotional executive performance in behavioural tasks of inhibitory control and emotion regulation (32).
6) Lower interpersonal stress and trait anxiety, higher self-esteem, and more friendship and family support (3, 55).
7) Finally, we expect that the neurocognitive mechanisms that facilitate resilient functioning to stress at in-unit assessment will be related to improved cognitive and emotional functioning at follow-up (lower rumination, lower interpersonal stress, improved mood) in line with the neuro-immune network hypothesis (24; Figure 3).

3. METHODS

3.1. Recruitment and eligibility

Recent research suggests that the link between CA and immune markers in the blood are small for hsCRP immune biomarkers in adults with depression (hsCRP r=0.15, 56). However, there are no studies investigating immune biomarkers in response to stress in adolescents. Therefore, it is difficult to determine the necessary sample size for our study. For instance, sample sizes for an intended power of 80% are r=0.15: n=345; r=0.2: n=193; r=0.3: n=84. Furthermore, it is well-established that sample correlations show fluctuations and are unstable in smaller samples. Simulation studies show that for a stable (i.e., replicable and generalizable) correlation, any sample would need to approach N=250 individuals (57). For this reason, to increase power to find small effects, we will recruit N=200 participants. With this sample size, our study is

appropriately powered to detect correlations from r>0.19.

Participants will be recruited from the general population and from previous studies conducted in the Department of Psychiatry of the University of Cambridge. First, we will contact participants from the Neuroscience in Psychiatry Network (NSPN) study who agreed to be contacted again. NSPN is a multi-centre accelerated longitudinal community cohort study (N=2389) focusing on normative adolescent to young adult development (between the ages of 14 and 24). Overall, this sample can be described as healthy, reporting low levels of psychopathological symptoms, behaviours and/or personality traits, and average mental wellbeing scores. From this cohort, we will contact those who agreed to be contacted for future research of whom family adversity scores were in the highest 25% of the entire NSPN cohort (≥75%=597 eligible individuals) as assessed by van Harmelen et al., 2017 (35). Family adversity scores in NSPN were calculated using a Principal Component Analysis (PCA) on standard-normally transformed sum scores for the Measure of Parenting Style (MOPS) and the Alabama Parenting Questionnaire (APQ) (see 35 for details). Eligible participants will be contacted via email first. This email will include the participant information sheet (PIS) of the study. A reminder email will be sent after a month. Finally, we will contact those participants who cannot be reached by email via phone call. To recruit participants from the general population we will distribute flyers and advertisements in colleges, Addenbrooke's hospital, and online. Individuals expressing an interest in the study could either email or telephone a member of the research team and leave their contact details. A member of the RAISE study research team will then phone interested individuals. During the telephone call a member of the research team will discuss the content of the PIS and assess the inclusion and exclusion criteria in order to ensure they are eligible and fully aware of the nature of the study. Eligible participants will be emailed the PIS of the study. Potential participants will be given the opportunity to raise any queries regarding any aspect of the study including confidentiality, anonymity, storage and use of data, as well as the right to withdraw.

The inclusion criteria will be: aged 16-26 years old; able and willing to give informed consent; able to speak, write, and understand English; BMI between 18.5 and 29.9 kg/m2; have experienced adverse life experiences and/or CA within the family environment (e.g., emotional, sexual and/or physical abuse, emotional and/or physical neglect, marital distress/conflict, parental mental health problems and/or parental

alcohol dependence, violence and/or aggressive behaviour) before the age of 16; and willing to abstain from strenuous exercise for 72 hours prior to the in-unit assessment.

The exclusion criteria will be: alcohol or substance use disorder within the past 6 months; current disorders likely to compromise the interpretation of the data (including, but not limited to, psychiatric disorders, immunological disorders, cardiovascular disorders, endocrine and autoimmune disorders, malignancies or infections, or any other condition to be determined by the principal investigator or delegate); current medication likely to compromise the interpretation of immunological data (including, but not limited to, corticosteroids or any other substance to be determined by the principal investigator or delegate); and contraindications to MRI (e.g., pacemaker or other implantable device or pregnancy).

3.2. Testing protocol and procedure

All included participants will be asked to complete 3 phases:

Phase I: an online assessment to assess psychological functioning and early life experiences; phase II: an in-unit assessment to assess neuro-immune and cognitive responses to stress, and phase III: an online follow-up assessment to assess psychological functioning after stress exposure. Please see Figure 4 and sections below for a description of the measures included in each phase.

Phase I: online assessment

The online assessment will include signing an informed consent form (ICF), the completion of a set of self-report questionnaires and two cognitive tasks online. The self-report questionnaires included in this assessment will be the Mood and Feelings Questionnaire (MFQ; 58), Revised Children's Manifest Anxiety Scale (RCMAS; 59), Leyton Obsessional Inventory (LOI; 60), the 10-item version of the Kessler Psychological Distress Scale (K10; 61), the Behaviours Checklist (BC), the Warwick-Edinburgh Mental Well-being Scale (WEMWBS; 62), the Measure of Parenting Style (MOPS; 63), the Alabama Parenting Questionnaire (APQ; 64), the Cambridge Friendship Questionnaire (CFQ), Family Assessment Device (FAD; 65, 66), Rosenberg Self-Esteem Scale (RSES; 67), Childhood Trauma Questionnaire (CTQ; 68) and the Drugs, Alcohol, and Self-Injury Inventory (DASI; 69). These questionnaires will be

used to calculate a composite measure of resilient functioning (as described in 35). The cognitive tasks will be the Emotional Stroop task (70) and Emotional Regulation task (71). More information about these measures is provided in the Supplementary Material.

Phase II: in-unit assessment

Following completion of the online assessment, participants will be contacted to schedule an appointment at Addenbrooke's Hospital (Cambridge, UK), after which they will receive a letter with the appointment details. This letter will include clothing and make-up regulations for brain scanning, time and location of the facilities where the evaluations will take place, as well as the research team's contact details. It will also reiterate that participation is voluntary and that participants can withdraw at any given time during the study. The second assessment will have a duration of 5 hours and will include (1) the completion of a second ICF, (2) a clinical evaluation, (3) a research nurse protocol, (4) an MRI session, and (5) the completion of a second set of self-report questionnaires. Please see the sections below for a description of the instruments included in each assessment.

Clinical evaluation

Participants will be asked to attend the clinical research facility (CRF) at Addenbrooke's Hospital at 9am, where they will be given a detailed overview of the study, asked to sign the ICF, and receive a low-fat breakfast adapted to their nutritional needs. The breakfast provided will exclude antioxidants such as glutathione and vitamins E and C to reduce the effects of these variables on the inflammatory markers addressed (72). Subsequently, the clinical evaluation will take place. This session will include the evaluation of psychiatric disorders, handedness, physical activity, sleep and eating patterns, medications (e.g., oral contraception), and intellectual functioning. Specifically, we will use the following measures: The Mini-International Neuropsychiatric Interview (MINI; 73), The Edinburgh handedness inventory (74), The Short-form Frequency Food questionnaire (SFFFQ; 75) and The Wechsler Abbreviated Scale of Intelligence (WASI; 76). The measures used will be supplemented with an interview. See the Supplementary Material for a description of the instruments used in this evaluation.

Physiological evaluation protocol

The clinical assessment will be followed by a physiological evaluation protocol. A research nurse will assess physical variables such as body temperature, height, weight, waist circumference, blood pressure (systolic and diastolic), and will implant a cannula to take blood samples during the assessment day.

Venepuncture

The implantation of the cannula will be conducted according to the standard Cambridge Clinical Research Facility protocol with risk protocols in place. These procedures include a brief interview about adverse experiences with blood assessment (such as fainting), as well as their preference for one arm or the other and if they want to lie down, or sit upright during the implantation of the cannula. Up to 30 mL of venous blood will be collected per participant for the measurement of cortisol, blood cytokines and immunophenotyping (i.e., basic immune cell counts and cell phenotyping). Blood will be extracted using an intravenous catheter inserted in the antecubital vein of the arm of the participants. 30 minutes following catheter insertion, the participants will undergo MRI scanning and will perform the psychological stress task (i.e., MIST). 1.2 mL K2 EDTA tubes will be taken for the analysis of immunophenotyping, 2.6 mL serum white tap tubes for the analysis of cytokines and 4.6 mL serum brown tap tubes for the analysis of cortisol. Bloods will be acquired at 4 time points: (-T1) 45 min before the start of the task (baseline line), (T0) right before the start of the task, (T30) right after the end of the task (peak cortisol) (T80) 80 min after the start of the task (delayed immune reactions) (81). Please see Figure 5 for a representation of the venepuncture protocol.

Neuroimaging protocol

Before the scan, participants will complete an MRI screening form, the State-Trait Anxiety Inventory (77), and practice the MIST. Each participant will be in the MRI scanner for about 50 minutes. The MRI scanning session will comprise the following MRI sequences:

T1-weighted three-dimensional magnetisation-prepared rapid gradient-echo (MPRAGE) (6 mins). High spatial resolution T1-weighted structural scans will be used

to aid normalisation and visualisation of each of the other MRI modalities (as described below) and analyse brain structure (i.e., cortical thickness, grey matter volume).

Resting state functional MRI (7 mins). A resting state fMRI will be used to investigate effects of inflammation on brain functional connectivity.

Montreal Imaging Stress Task (30 mins). We will use a modified version of the Montreal imaging stress task (MIST; 78). This task comprises a series of computerized mental arithmetic tasks with an induced failure component. The protocol consists of a training session conducted outside the imaging unit, and a test session during which the functional images are acquired. Please see the Supplementary Material for a description of the paradigm.

After the MRI session, participants will be debriefed. We will tell them that the task was designed to be impossible to accomplish and that it did not truly assess their ability to perform mental arithmetic. Then, we will ask them to complete the STAI-State, MFQ, Life Events Questionnaire (79), and any questionnaire from the online assessment that is incomplete. After completion of the post-MRI session, the participants will have a standard meal and be given time to relax. In addition, we will have a protocol for debriefing, an information letter with relevant types of support available, and a distress protocol in case the participant reports severe distress.

Phase III: follow-up online assessment

The follow-up and final assessment will be completed online within a month from the in-unit assessment and will include the signature of the third and last online ICF, the MFQ, RCMAS, and LEQ, as well as the following measures described in the Supplementary Material: Perceived Stress Scale (PSS; 80), Interpersonal Sensitivity Measure (IPSM; 81) and the 10-item Ruminative Response Scale (RRS-10; 82).

Please see the Supplementary Material for a relation of the main risk and ethical issues associated with this protocol.

4. ANALYSES

Clinical, questionnaire, and immunological data will be descriptively summarised. The significance threshold will be set at $p<0.05$ and Family-Wise Error (FWE) corrections

will be applied to correct for multiple comparisons.

4.1. Preprocessing

Quantification of resilient functioning

Using the data collected during the first online assessment, we will calculate gender and age-related degree of resilient functioning based on the model by van Harmelen et al. (35). Specifically, we will conduct a PCA on standard-normally transformed individual total scores on the MFQ, RCMAS, LOI, BC, SES, K10 and WEMWBS. From this analysis, we will extract individual scores for the first component to reflect individual current psychosocial functioning scores. We will similarly conduct a PCA on standard normal transformed MOPS, CTQ and APQ data to establish a single score that reflects CA. Next, we will relate the psychosocial functioning component score against the CA score (35). From this model, we will extract the residual scores as a measure of individual degree of resilient functioning: the extent to which an individual has better, or worse, psychosocial functioning than the average score expected given their CA experiences (35). For parsimony, we will refer to this as degree of 'resilient functioning' with higher scores reflecting better (conditional) psychosocial functioning. These individual resilient functioning scores will be utilized in the analyses described below.

Imaging preprocessing

Task-evoked and resting state fMRI data will be preprocessed using Statistical Parametric Mapping 12 (SPM12) (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) implemented in MatLab R2018b (Mathworks, Natick, MA, USA). Images will be corrected for movement artefacts, coregistered with the T1-weighted images, normalised to a standard EPI template in the Montreal Neurological Institute (MNI) space and spatially smoothed with an 8 mm FWHM Gaussian kernel. Quality control will be performed after each pre-processing step. Structural MRI will be analysed using the FreeSurfer image analysis suite, which is widely documented and freely available online (http://surfer.nmr.mgh.harvard.edu/).

Cortisol and immune markers preprocessing

Blood samples will be processed at the Core Biochemical Assay Laboratory (CBAL) (blood cytokines), Pathology (cortisol), and Immunology laboratory

(immunophenotyping) at Addenbrookes Hospital (Cambridge, UK). 1ml of blood will be diluted 1:1 with Phosphate Buffered Saline (PBS) and stimulated with lipopolysaccharide (LPS) (LPS challenge) to analyse the production of IL-6 and other cytokines difficult to detect (83, 84). LPS-stimulated IL-6 and IL-1β production in peripheral leukocytes will be processed following a protocol originally developed by DeRijk et al. (85). All cytokines, including serum TNF-α and hs-CRP will be measured using the MSD platform.

Phenotyping

50-100 ul of blood will be used for the enumeration of the major populations of immune cells (monocytes, granulocytes, NK cells, NKT cells, CD4+ and CD8+ T cells, and B cells). We will use multi-colour flow cytometry to count and analyse the size, shape, and properties of individual cells within heterogeneous populations. We will use multivariate methods (partial least squares and PCA) to reduce dimensionality and define populations of differentially co-expressed cell counts and the unbiased gating algorithm SPADE (Spanning-tree Progression Analysis of Density-normalized Events). SPADE generates an immune cell hierarchy by clustering phenotypically similar cells into groups which can be enumerated.

4.2. Statistical analyses

Age, gender, socioeconomic status, and education level will be included as covariates in all analyses. Additionally, for the imaging analysis we will include the total volume of grey matter (for the analyses of grey matter), a high-pass filter uses to remove low-frequency drifts in the data (for the analysis of fMRI data), and the signal fluctuations in white matter and cerebrospinal fluid and the subject-specific 6 realignment parameters and their first order derivatives (for the analyses of functional connectivity). Finally, phase of menstrual cycle, BMI, and tobacco smoking will be used in the analyses involving immune/cortisol markers.

Hypothesis 1: Individuals with higher resilient functioning will display lower baseline cortisol and blood cytokines, faster habituation, and less cortisol volatility:

We will use a PCA to derive a factor score for endocrine or inflammatory markers at the different time points. We expect there will be two components: one relating to baseline

cortisol (or index of immune biomarkers), and one relating to cortisol *change* (or index of immune responsivity; see 86 for a similar approach). We will then utilise area under curve (AUC) analyses to examine whether degree of resilient functioning is more strongly associated with general immune status (i.e., the baseline cortisol measure) or cortisol responsivity (i.e., area under the curve with respect to ground (AUC_G) and area under the curve with respect to increase (AUC_I) (see 87 for specifics). We will validate this result using a simple multivariate regression to examine whether the two components of immune functioning independently predict resilient functioning outcomes.

Hypotheses 2-4: Higher resilient functioning is associated with more balanced and integrated neural systems:

Imaging data will be analysed using well-established methods for quantification of structural and functional parameters. We will use both whole-brain and regions of interest (ROIs) approaches. We will use the MPFC, ACC, amygdala, insula, and hippocampus as ROIs. We will conduct multiple regression analysis to test the relationships between brain imaging and the variables of interest. The significance threshold will be set at $p<0.05$ after family-wise error correction for multiple comparisons across the whole-brain ($pFWE<0.05$) or the voxels of the different regions of interest ROIs (i.e., using small volume correction [SVC] procedures [$pFWE\text{-}SVC<0.05$]).

Hypothesis 5 & 6: Individuals with higher resilient functioning will display better cognitive control and greater levels of social support.

We will use Structural Equation Modelling (SEM) to examine the relations and interrelations described in hypotheses 5 & 6. Specifically, we hypothesise that, using a multiple indicators multiple causes (MIMIC) model, we will observe that individual differences in outcome (cognitive functioning) will be explained by partially independent and complementary neural systems (i.e., all paths shown will be significant when estimated simultaneously).

Hypothesis 7: Neurocognitive mechanisms that facilitate resilient functioning to stress are related to improved cognitive and emotional functioning at follow up.

Using mediation modelling (e.g. Figure 2), we will examine whether the mechanisms that facilitate resilient functioning to stress are related to improved cognitive and emotional functioning (i.e., rumination, interpersonal stress, mood, etc.) at follow up.

Immunophenotyping analyses

We will assess immunophenotyping data to determine whether or not resilient adolescents can be distinguished in terms of immunophenotype levels. The proportion of each immune cell type will be used as the predictor variables in a Partial Least Squares (PLS) analysis, with resilient functioning as the response variable. Using this method, we hope to identify immune patterns that are associated with resilient functioning.

5. CURRENT STATUS

We are currently at year 3 of the project. As of March 2020, 102 participants have been recruited for the study. From those, 62 have completed phases I, II and II of the study. Only ten participants have withdrawn following informed consent due to various reasons (e.g. scheduling conflicts, presence of mental health disorders or found images presented in the Emotional Regulation Task overly distressing). Additionally, of the adolescents screened, the most common reasons for ineligibility are MRI incompatibility (e.g. dental braces), current psychotropic medication, and BMI outside 18-30. Due to the uncertainty caused by the COVID-19 outbreak we cannot anticipate when we will be able to finish the recruitment of our participants. However, we are approaching community organizations and agencies across Cambridge, including agencies working with victims of trauma to aid in the recruitment of participants with CA, and therefore we anticipate the finalization of the recruitment in three months from the time we start recruiting again.

6. DISCUSSION

The RAISE study aims to examine how resilient adolescents react to psychosocial stress in order to better understand the neurobiological mechanisms that contribute to adolescent resilience. We will examine how key biological systems (i.e., the HPA axis and the immune system) interrelate and interact with brain function and social influences in order to facilitate resilient functioning after CA. We hypothesize that

resilient functioning would be facilitated by reduced baseline cortisol and cytokine levels before and after the stressor (i.e., a latent factor constructed by serum hs-CRP and TNF-α and IL-6 and IL1β levels) as these have been shown to be increased in those with CA, mental illness, reduced stress related brain responses (i.e., amygdala, insula), and increased modulatory responses in the MPFC and ACC during the MIST. Moreover, we expect to find that the neurocognitive mechanisms that facilitate resilient functioning to stress during the in-unit assessment will be related to improved cognitive and emotional functioning at follow-up (lower rumination, lower interpersonal stress, improved mood). The findings from this study will help us to determine what sets resilient individuals apart on a neurobiological level. Such knowledge will be helpful to inform intervention strategies for individuals with a history of CA to prevent the development of mental health disorders, and ultimately increase resilience in individuals who have experienced adversity in early life.

DATA SHARING

Data will be available at the University of Cambridge Repository and accessed upon reasonable request.

AUTHOR CONTRIBUTIONS

Dr. Laura Moreno-López lead the recruitment and testing of participants and drafted the manuscript. Ms. Samantha N. Sallie, Konstantinos Ioannidis, Muzaffer Kaser, Katja Schueler, Adrian Dahl Askelund and Lorinda Turner were instrumental to the design of the study and reviewed the manuscript. The RAISE consortium members were or are instrumental to the setup and/or assessment of the RAISE study. Dr. Anne-Laura van Harmelen conceptualized and designed the study, drafted the original grant proposal, obtained financial support, oversaw all study procedures, and reviewed and revised this manuscript. All authors approved the final manuscript and agree to be accountable for all aspects of the work presented.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest associated with this project.

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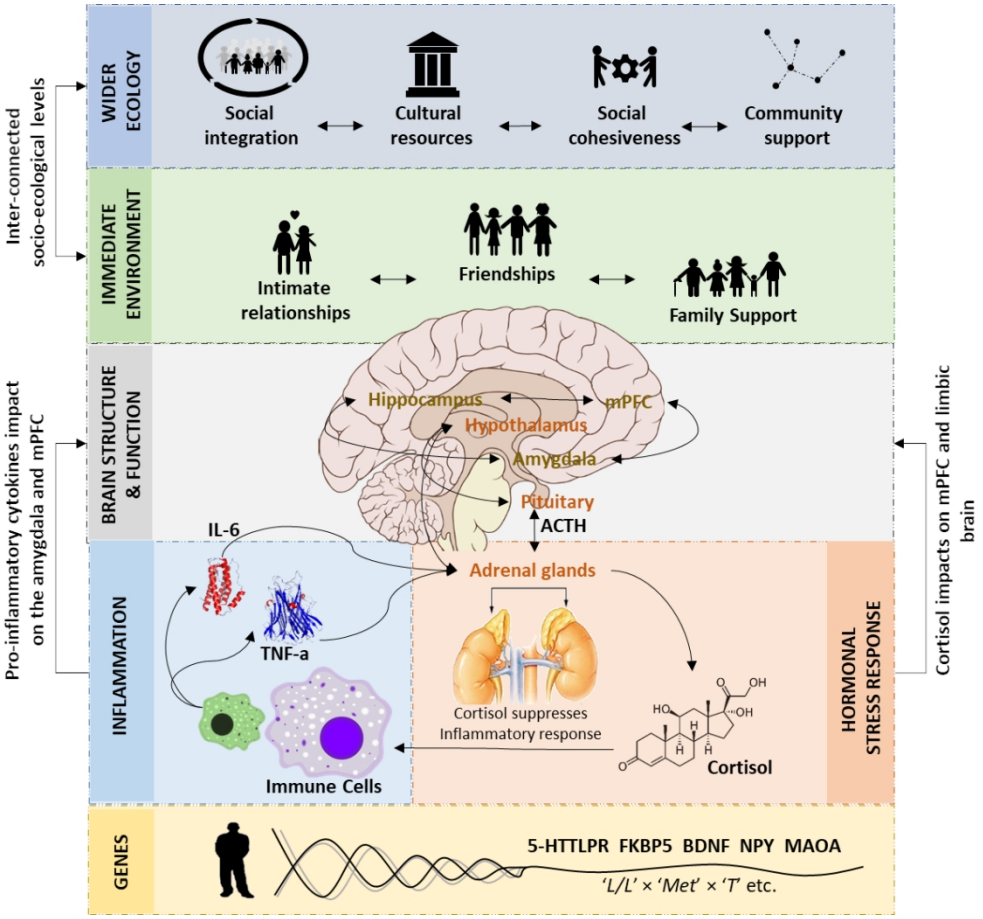

Figure 1. The complex neurobiology of resilience after childhood maltreatment (CM) (36). Resilient functioning in those individuals who have experienced CM may be facilitated by larger prefrontal cortex (PFC) and hippocampal volume and connectivity, the ability to adequately regulate emotions and dampen stress responsivity, cortisol and proinflammatory baseline and responses, polygenic resilience effects, social support from the immediate environment, and the wider ecology. For readability, the location of the hippocampus is not correct. 5-HTTLPR serotonin-transporter-linked polymorphic region, ACTH adrenocorticotropic hormone; BDNF brain-derived neurotrophic factor, FKBP5 FK binding protein 5, IL-6 interleukin 6, MAOA monoamine oxidase A, mPFC medial PFC, NPY neuropeptide-Y, TNFα, tumour necrosis factor-α.

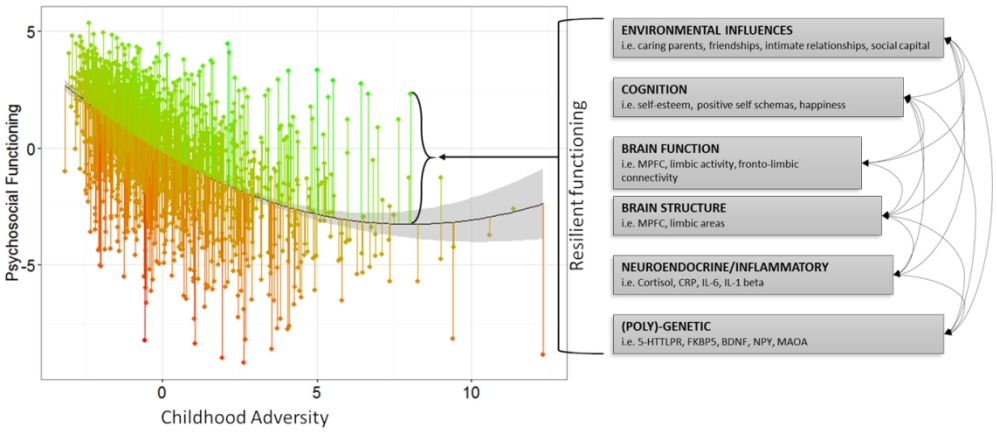

Figure 2. Risk to Resilient functioning in the NSPN sample of N=1980 adolescents (35). Green and red lines indicate functioning that is better or 'resilient' (green) or worse 'vulnerable' (red) than expected. Psychosocial functioning reflects a factor score (mean=0, SD=1) derived from multiple measures of psychiatric symptomatology, personality traits, and mental wellbeing. CA reflects a factor score (mean=0, SD=1) from two measures which assess early life family experiences.

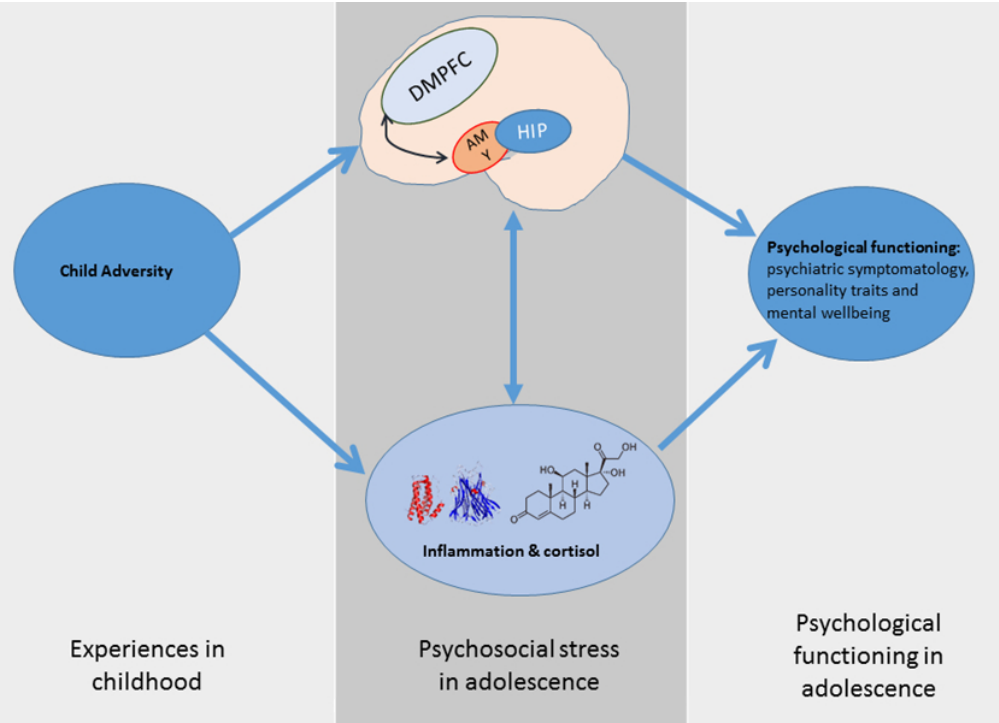

Figure 3. The neuro-immune network hypothesis of child maltreatment. Inflammatory protein and brain responses to psychosocial stress mediate the relationship between CA and psychological functioning.

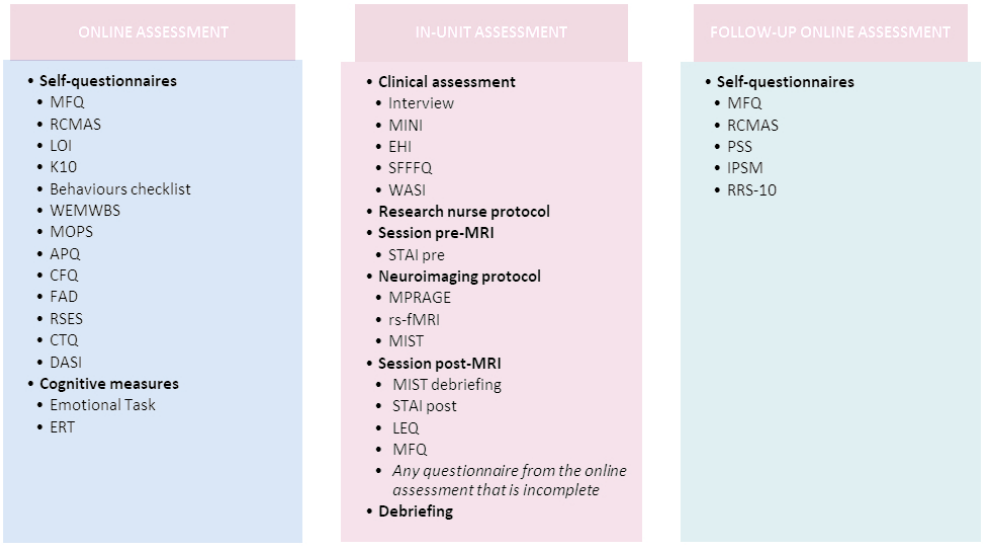

Figure 4. Summary of the study protocol. Abbreviations: APQ=Alabama Parenting Questionnaire, CFQ=Cambridge Friendship Questionnaire, CTQ=Childhood Trauma Questionnaire, DASI=Drugs Alcohol and Self-Injury, EHI=Edinburgh Handedness Inventory, ERT=Emotion Regulation Task, FAD=Family Assessment Device, IPSM=Interpersonal Sensitivity Measure, K10=Kessler Psychological Distress Scale, LEQ=Life-Events Questionnaire, LOI=Leyton Obsessional Inventory, MFQ=Mood and Feelings Questionnaire, MINI=Mini-International Neuropsychiatric Interview, MIST=Montreal Imaging Stress Task, MOPS=Measure of Parenting Style, MPRAGE=T1-Weighted rapid three-dimensional radient-echo, PSS=Perceived Stress Scale, RCMAS=Revised Children's Manifest Anxiety Scale, RRS-10=Ruminative Response Scale, RSES=Rosenberg Self-Esteem Scale, rs-fMRI=Resting state functional magnetic resonance imaging, SFFFQ=Short-Form Frequency Food Questionnaire, STAI=State-Trait Anxiety Inventory, WASI=Wechsler Abbreviated Scale of Intelligence, WEMWBS=Warwick-Edinburgh Mental Well-Being Scale.

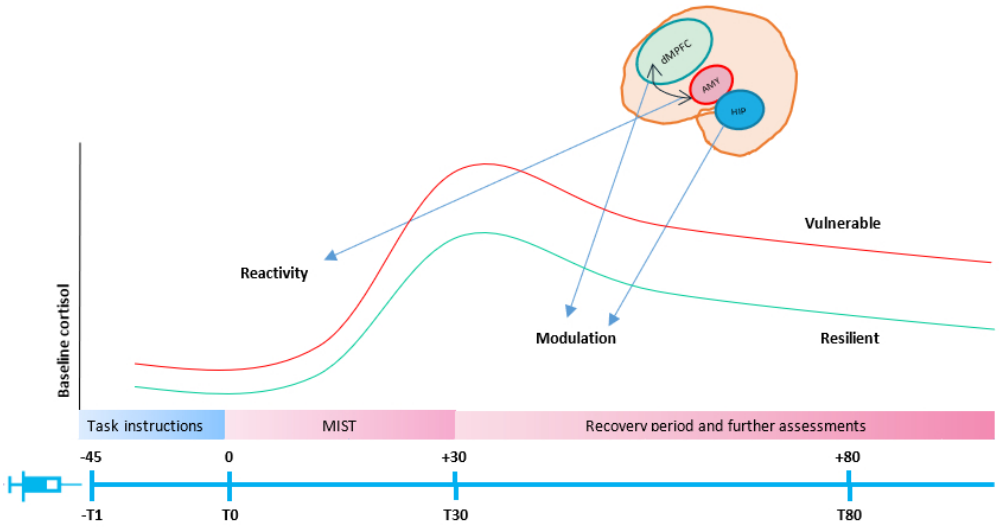

Figure 5. Venepuncture protocol. Bloods will be acquired at 4 time points: (-T1) 45 min before the start of the task, (T0) Just before the start of the task (T30) right after the start of the task, (T80) 80 min after the start of the task.

Supplementary Material

The RAISE study protocol; a cross-sectional, multi-level, neurobiological study of studying resilience after individual stress exposure

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Contents

Measures used in Phase I

Psychological functioning

Mood and Feelings Questionnaire. The Mood and Feelings Questionnaire (MFQ; Angold et al., 1995) is a 33-item instrument that was developed to measure depressive symptoms (over the course of two weeks prior and up to the date of the assessment) in children and adolescents from 8- to 18-year-olds. The MFQ has shown prognostic validity in clinical and non-clinical samples (Daviss et al., 2006; Wood et al., 1995). Higher sum scores indicate more symptoms.

Revised Children's Manifest Anxiety Scale. The Revised Children's Manifest Anxiety Scale (RCMAS; Reynolds & Richmond, 1978) is a 37-item questionnaire that includes three anxiety factors (physiological anxiety, worry/oversensitivity, and social concerns/concentration), as well as a total anxiety score. A higher score indicates a high level of anxiety.

Leyton Obsessional Inventory. The Leyton Obsessional Inventory (LOI; Bamber et al., 2002) is an 11-item questionnaire that measures obsessional/anxiety symptoms. Responses ranged from 'always,' 'mostly,' 'sometimes,' to 'never.' Higher scores indicate more obsessions.

Kessler Psychological Distress Scale. The 10-item version of the Kessler Psychological Distress Scale (K10; Kessler et al., 2002) measures frequency of nervousness, hopelessness, sadness, worthlessness, and fatigue. Responses are summed to create a total score, with higher scores signifying more psychological distress.

Behaviours checklist. The Behaviours Checklist (BC) is an 11-item questionnaire for symptoms of antisocial behaviour based on the Diagnostic and Statistical Manual (DSM-IV) conduct disorder definition. This measure has not been previously published but the results of previous analysis showed that its internal consistency was good at baseline (Cronbach's alpha=0.74) (van Harmelen et al., 2017). A high score indicates greater emotional and behavioural problems.

Warwick-Edinburgh Mental Well-being Scale. The Warwick-Edinburgh Mental Well-being Scale (WEMWBS; Tennant et al., 2007) was developed to enable the monitoring

of mental wellbeing in the general population; and the evaluation of projects, programs, and policies aiming to improve mental wellbeing in the UK. It comprises 14 positively worded statements with five response categories from 'none of the time' to 'all of the time.' Higher scores indicate better mental well-being.

Cambridge Friendship Questionnaire. The Cambridge Friendship Questionnaire (CFQ) is an 8-item questionnaire assessing the number, availability, and quality of friendships. Higher scores indicate better perceived overall quality of friendships. The CFQ has good measurement invariance and external validity, and has demonstrated ecological validity across two samples (van Harmelen et al., 2016).

Family Assessment Device. The Family Assessment Device (FAD; Epstein et al., 1983; Miller et al., 1985) is a 12-item scale measuring overall functioning of the family. Six items describe healthy functioning and the other six describe unhealthy functioning. Each item is rated on a 4-point Likert scale (4='strongly agree,' 3='agree,' 2='disagree,' 1='strongly disagree'). The higher the score the worse the family functioning.

Rosenberg Self-Esteem Scale. The Rosenberg Self-Esteem Scale (RSES; Petersen, 1975) is a 10-item scale that measures positive and negative feelings of self-worth. All items are answered using a 4-point Likert scale format ranging from strongly agree to strongly disagree. Lower scores indicate lower self-esteem.

Childhood Trauma Questionnaire. The Childhood Trauma Questionnaire (CTQ; Bernstein et al., 1994) is a standardised, retrospective 28-item self-report inventory that measures the severity of different types of childhood trauma, producing five clinical subscales: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. Participants respond to each item in the context of 'when you were growing up' and answer according to a 5-point Likert scale ranging from 1='never' to 5='very often.' The score ranges from 5 to 25 for each subscale, with scores falling into four categories: none to low trauma exposure, low to moderate trauma exposure, moderate to severe trauma exposure, and severe to extreme trauma exposure. The measure also includes a 3-item minimisation/denial scale indicating the potential underreporting of maltreatment.

Drugs Alcohol and Self-injury. The drugs, alcohol, and self-injury inventory (DASI; Cassels et al., 2018) is a 14-item self-report measure of cigarette, alcohol, and drug use, and non-suicidal self-injury.

Child adversity

Measure of Parenting Style. The Measure of Parenting Style (MOPS; Parker et al., 1997) is a questionnaire designed to assess different approaches to parenting during the first 16 years of life. Participants have to evaluate each parent based on 15 statements (e.g., 'overprotective of me' and 'uncaring of me') using a four-point scale, yielding total scores for each parent on subscales labelled 'indifference,' 'abuse,' and 'over-control.' The sum scores of items in each category indicate the degree to which that parenting style was experienced by an individual.

The Alabama Parenting Questionnaire. The Alabama Parenting Questionnaire (APQ; Elgar et al., 2007) measures parenting practices. Participants are asked to rate how frequently each behaviour occurred in their family home on a 5-point scale ranging from 'never' to 'always.' High scores can indicate positive (i.e., involvement) or negative (i.e., inconsistent discipline, poor supervision, corporal punishment) parenting.

Cognitive functioning

Emotional Stroop task. In this task, participants have to categorise an adjective as angry, happy, or sad while ignoring the valence of the expression on a face upon which the adjective is superimposed (Preston & Stansfield, 2008). There are three blocks of trials: words only (32 trials), faces only (32 trials), and words and faces combined (64 trials). In the word-only block, the stimulus is one of the four emotion words (HAPPY, SAD, ANGRY, or SCARED; 8 trials each). In the face-only block, the stimulus is 1 of 16 pictures of facial affect (Ekman & Friesen, 1976), each appearing twice. The 16 pictures consist of 1 picture displaying each of the four emotions (happy, sad, angry, and scared) from each of four actors (older man, younger man, blonde woman, and brunette woman). In the word-and-face block, the stimulus is 1 of the same 16 faces with one of the four emotion words superimposed semi-transparently (89% transparent) over the face, centred vertically on the nose. On 48 trials in the word-and-face block, the facial expression matched the word (congruent trials—12 repetitions of each word matched to three iterations of each of the four actors expressing that emotion). On the other 16

trials, the facial expression did not match the word (incongruent trials—4 repetitions of each word matched at random to one of the possible remaining incongruent facial expressions). The emotional Stroop task generates two indices of affective executive control: the incongruency index and the congruency index. The incongruency index is the cost in reaction time to correctly categorize an emotional adjective when the background face depicts an incongruent emotional expression relative to when the face depicts a neutral emotional expression. The congruency index reflects the facilitation in reaction time to categorize an emotional adjective when the background face depicts a congruent facial expression relative to the neutral condition.

Emotion Regulation task. This task assesses the use of two strategies of cognitive emotion regulation: reappraisal and attentional control (i.e., distraction) (Kanske et al., 2011). Participants view neutral or negative emotional pictures on the screen one by one. After a short time, a text instruction appears on the screen to either 'View,' 'Reappraise,' or 'Distract' the emotion elicited by the current picture. Each picture appears in nearly every condition for approximately 6 seconds (in total, 128 trials). The 'View' condition refers to not regulating the emotional response to the picture at all. 'Reappraisal' means attenuating the initial emotional response by finding alternative interpretations of the picture. During 'Distraction' trials, a simple arithmetic task appears on the screen to which participants can then shift their attention in order to downregulate their emotions. After each trial, participants rate their current emotional state using the Self-Assessment Manikin (SAM). This task provides indices of individual general emotional reactivity, reappraisal effects and distraction effects. Scores are derived from rating differences between emotional categories and emotion regulation conditions.

Measures used in Phase II

Clinical evaluation

Mini-International Neuropsychiatric Interview. The Mini-International Neuropsychiatric Interview (MINI) was developed by Sheehan and Lecrubier (Sheehan et al., 1998) to meet the need for a brief, reliable, and valid structured diagnostic interview. The MINI contains 130 questions that assess 16 axis I DSM-IV disorders and is organised in diagnostic modules. For most modules, two to four screening questions

are used to rule out the diagnosis when answered negatively. Positive responses to screening questions are explored by further investigation of other diagnostic criteria (according to the standard MINI assessment protocol).

Edinburgh Handedness Inventory. The Edinburgh handedness inventory is an instrument used to assess the dominance of a person's hand in everyday activities (Oldfield, 1971). We will use the self-administered version.

Short-form Frequency Food Questionnaire. The Short-form Frequency Food questionnaire (SFFFQ; Cleghorn et al., 1016) is a measure of the quality of dietary habits. It evaluates the consume of 25 items including fruit, vegetables, fibre-rich foods, high fat and high-sugar foods, meat, meat products, and fish. The participants are asked to tick one option (ranging from 'rarely' to '5+ a day') for each of the items.

Wechsler Abbreviated Scale of Intelligence. The Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) is a short measure of IQ composed by four subtests: Vocabulary, Block Design, Similarities and Matrix Reasoning. We will use the subtests Vocabulary and Matrix reasoning to estimate the IQ of each participants.

Neuroimaging protocol

The State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983) is a measure of state and trait anxiety that includes items such as: 'I am tense,' 'I am worried,' 'I feel calm,' and 'I feel secure.' All items are rated on a 4-point scale (e.g., from 'Almost Never' to 'Almost Always'). Higher scores indicate greater anxiety. Internal consistency coefficients for the scale have ranged from 0.86 to 0.95.

Montreal Imaging Stress Task (30 mins). We will use a modified version of the Montreal imaging stress task (MIST; Dedovic et al., 2005). This task comprises a series of computerized mental arithmetic tasks with an induced failure component. The protocol consists of a training session conducted outside the imaging unit, and a test session during which the functional images are acquired. Please see the Supplementary Material for a description of the paradigm. The test session has two runs. Each run has three conditions: rest, control, and experimental. During the experimental session, the program is set to a time limit that is 10% less than the subject's average response time recorded during the training session. This approach induces a high failure rate. In

addition, the program continuously records the participant's average response time and the number of correct responses. If the participant answers a series of 3 consecutive mental arithmetic tasks correctly, the program reduces the time limit to 10% less than the average time for the 3 correctly solved tasks. Conversely, if the subject answers a series of 3 consecutive tasks incorrectly, the program increases the time limit for the following tasks by 10%. As such, under experimental conditions, a range of about 20% to 45% correct answers is enforced (Figure S1). Moreover, between experimental runs, the participant will see a screen with information about their performance, reminding them that the average performance is about 80%–90% correct answers and that they must improve their performance. During the control condition, mental arithmetic is presented with the same level of difficulty and at the same frequency as during the experimental sessions, but no time restriction is enforced, and individual performance and average users' performance are not displayed. Feedback (“correct” or “incorrect”) is still shown after each task, but because of the absence of a time limit, average performance increases to about 90%. Finally, during the rest condition, the interface of the computer program remains on the screen, but no tasks are shown. After the first run, a member of the radiographer team will show disappointment about the participant’s performance and ask him/her to try harder (e.g., “You are not doing as well as we had hoped”, “Please remember that your performance needs to be close to the average to allow us to use your data”, “Let’s try it again” etc.).

The Life-Events Questionnaire evaluates recent undesirable and desirable life events and friendships (LEQ; Goodyer et al., 1997). Participants are asked to rate 13 major life events during the preceding 12-month period that may have affected them. These may include changes in school, deaths, household disasters, friendship difficulties, and illnesses. Respondents are asked how they felt about the event on a scale of 1=‘very pleasant/happy’ to 5=‘very unpleasant/sad/painful.’ A quantitative estimate of the adverse life events is obtained by summarising the number of events rated either 4 or 5 for more than two weeks.

Measures used in Phase III

Perceived Stress Scale. The Perceived Stress Scale (PSS; Cohen et al., 1983) is the most widely used psychological instrument for measuring the perception of stress. This 10-item scale measures the degree to which situations in one’s life are appraised as

stressful. Items are designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes direct queries about current levels of experienced stress. Scores can range from 0-40 with higher scores indicating higher perceived stress.

Interpersonal Sensitivity Measure. The Interpersonal Sensitivity Measure (IPSM; Harb et al., 2002) is a 28-item scale that assesses excessive sensitivity to the interpersonal behaviour of others, social feedback, and (perceived or actual) negative evaluation by others. The measure includes a total score and three subscale scores: interpersonal worry and dependency (eleven items; e.g., 'I worry about what others think of me'), low self-esteem (ten items e.g., 'If other people knew what I am really like, they would think less of me'), and unassertive interpersonal behaviour (eight items; e.g., 'I find it hard to get angry with people').

Ruminative Response Scale. The 10-item Ruminative Response Scale (RRS-10; Treynor et al., 2003) is one of the most widely used self-report measures of rumination, comprising 10 items and describing the factors of brooding and reflection. Each item is rated on a 4-points Likert scale ranging from 1='never' to 4='always'). The total score ranges from 10 to 40, with higher scores indicating higher degrees of ruminative symptoms.

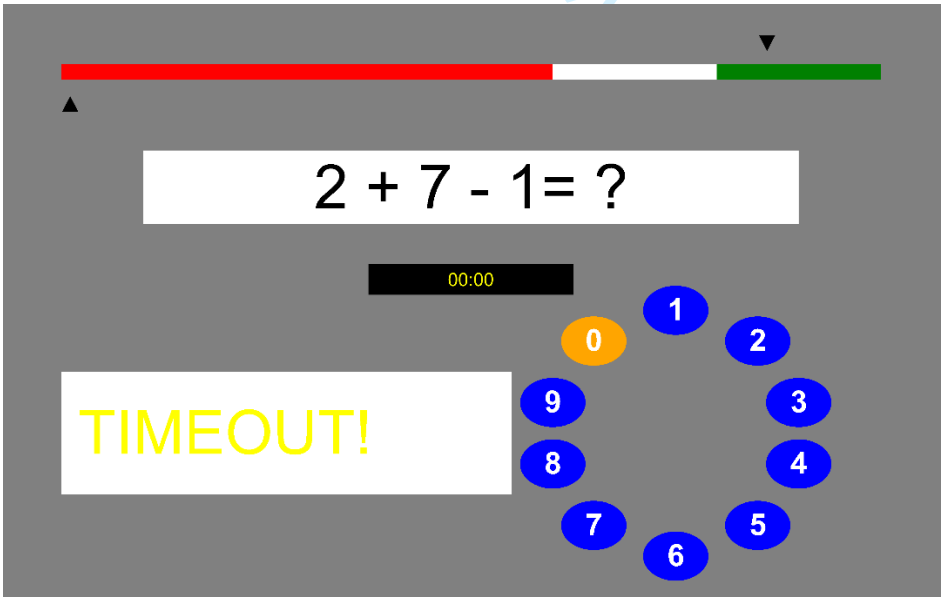

Figure S1. Graphical user interface of the MIST. From top to bottom, the figure shows the performance indicators (top arrow=average performance, bottom arrow=individual subject's performance), the mental arithmetic task, the progress bar reflecting the imposed time limit, the text field for feedback, and the rotary dial for the response submission.

Risk and ethical issues

The main risk and ethical issues associated with the protocol are: 1) Consent, 2) Confidentiality, 3) Risks and costs of participation, and 4) Compensation for participation.

Consent

Our participants will be 16 years old or older. We will obtain informed consent for each of the phases of the study: first, we will obtain consent verbally over the telephone when participants express their interest in the study. We will go through the PIS and inclusion/exclusion criteria to ensure participants are aware of the nature of the study. Participants will have opportunities to ask questions and details regarding confidentiality, anonymity, storage and use of data, as well as right to withdraw. Only those participants who meet the inclusion criteria and are willing to participate in the study will be invited to do so. We will send them an email with the links and instructions to complete the first online ICF, a set of self-rate questionnaires, and three cognitive tasks online. On the day of the scanning we will obtain informed consent in writing before the participants start the assessments. We will ensure that consent is voluntary and that participants are fully informed by asking whether they have understood everything on the form and whether they have any questions regarding the different assessments. We will then reiterate that participation is voluntary and that they can stop the evaluations at any time. Finally, and within a month of their in-unit evaluation at Addenbrooke's Hospital, we will send them an email with the link to complete the last online ICF and a set of self-rate questionnaires.

Confidentiality

Identifiable data will be linked by a unique ID number to the participant's anonymised study assessments. Participants will be carefully monitored to ensure that the procedures are followed in accordance with the UK Data Protection Act 2018. Only RAISE study team members, who are fully aware of their responsibilities to conform to the Data Protection Act 2018, will be allowed access to the personally identifiable data which will be stored in a highly secure, password encrypted database.

The transfer and storage of the participant's biological samples (venous blood) will be appropriately handled by our collaborators according to their standard operating procedures. All tissue samples will be labelled with a non-identifiable participant ID, date of birth, data of collection and sample identifier (i.e., serum), and stored in a -80° Celsius freezer at Addenbrookes Hospital. All samples will be scanned into a secure electronic database (i.e., RedCAP) to track their location and to ensure that all samples are accounted for.

All participants will be asked to provide consent to contact their parents (<18 year old participants) or general practitioner, in case the collected data suggest that the participant might require further clinical assessment or treatment.

Risks and costs of participation

Distress during the completion of the questionnaires and cognitive tasks online: some participants may find the questionnaires (e.g., CTQ, MFQ) or the cognitive tasks (e.g., emotion regulation task) distressing. In order to minimise this emotional burden we have made sure that both the questionnaires and cognitive tasks included in the study reiterate that the participant may withdraw at any time during the completion of the questionnaires/tasks, in which case, they will see a screen with a list of local mental health resources with activated web links. Moreover, following the completion of the Emotional Regulation Task, participants will be debriefed to inform them that the task was designed to produce an emotional response and it is completely normal and even intended that they reacted emotionally to the pictures presented.

Self-injury and suicidality disclosure: self-injury and suicidality disclosure will be addressed during the completion of the questionnaires online and at the end of the in-unit assessment. The completion of the questionnaires is configured in such a way that the RAISE study research team will receive an immediate email if the participant discloses current self-injury or suicidality (i.e., affirmative answer on Q19 of the MFQ and/or Qs11-12, or Qs14-15 of the DASI). The principal investigator and/or a suitable member of the research team will then review the participant's questionnaires with the psychiatrists affiliated with the study and if there is concern about imminent risk of self-injury or danger to the participant (e.g. disclosed current physical, sexual, or emotional abuse), they will call the participant. This will also be an opportunity to gather further

details (particularly regarding suicidal thoughts) and clarify whether they are in need of, and seeking, the appropriate help. If the risk is imminent, we will suggest the participant call First Response Service (111) or attend E&A. If the risk is not imminent and the participant is 18+ we will suggest they talk to their GP. If the participant is 16-17, we will suggest they talk to their GP and their parents/the people they live with about how they are feeling. If the participant refuses to seek help, we have a duty to care and it will be necessary to breach confidentiality. If the participant is 18+ we will contact their GP. If the participant is 16-17 we will contact their parents/guardians first. If contacting parents/guardians is contraindicated because of poor guardians-participant relationships (e.g., when disclosing to a parent may increase risk of suicide), direct contact with the participant's GP, or other local clinic or clinical support will be sought. Important points to note during disclosure to parents/guardians include: explaining that the study measures are not clinical instruments and thus cannot be used to detect future risk with absolute certainty, expressing concern about their child's responses to specific items, reinforcing that the safety of their child is of primary importance, helping them to think about how to get a psychological evaluation of their child and encouraging them to do so, reminding them that any information shared by their child was difficult for them to disclose, and recommending non-punitive and sensitive behaviour towards their child with regard to the issue. In the in-unit assessment, in addition to having lunch and time to relax, we will have a distress protocol in place in case the participant reports severe distress during or after the assessments.

Risks associated with the venepuncture: to minimise discomfort, blood taking will be conducted by a clinical research nurse, according to the standard Clinical Research Facilities operating procedures and risk protocols. The participant will have time to rest after the procedure.

Distress and discomfort during brain scanning: the MIST task is intended to be stressful. Therefore, after the session, we will debrief our participants by explaining to them that the task was designed to be impossible to accomplish and that it did not assess their true ability to perform mental arithmetic. Moreover, MRI scanners are very loud. In order to reduce any potential discomfort, all participants will be given earplugs for aural protection. MRI scanning also requires participants to lie still in the scanner which some may find uncomfortable or may induce feelings of claustrophobia. We will ensure that

participants are as comfortable as possible by providing neck and arm pillows. Furthermore, we will make sure that participants can communicate with a member of the research team at any time during scanning. They will be informed that if they would like to stop they may do so by pressing a button which will be easily accessible to them at all times.

Potential clinical findings: we will obtain consent from participants to inform their GP if any clinically relevant information comes to light during their participation in the study. In all cases, we will discuss it with a specialist and contact the participant first. If the participant refuses to seek help, we have a duty to care and it will be necessary to breach confidentiality. For neuroimaging findings, the Wolfe Brain Imaging Center (where the acquisition of the images will take place) policy is that all studies will include at least T1 and T2 weighted datasets, that are internally reported by a clinically qualified reviewer, who will refer to the WBIC clinical lead. The clinical lead will counsel the individual regarding further clinical referrals (including potential GP referral).

Time burden: the online assessments are not expected to last more than 2 and 1 hours respectively. During the in-unit assessment each participant will be tested for no longer than 5 hours in total. These durations will be clearly communicated to the participants at the beginning of the study.

Compensation for participation

To compensate for their time, participants will be paid £150. In addition, breakfast and lunch will be provided on the in-unit assessment day.

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The RAISE study protocol; a cross-sectional, multi-level, neurobiological study of studying resilience after individual stress exposure

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ABSTRACT

Introduction: This paper describes the protocol for an ongoing project funded by the Royal Society, the Resilience After Individual Stress Exposure (RAISE) study; which aims to examine the factors and mechanisms that facilitate resilient functioning after childhood adversity (CA). **Methods and Analysis:** We aim to recruit up to 200 participants. We will use dimension reduction techniques (PCA) on standard-normally transformed individual parameters of mental health, social functioning and CA to calculate a composite measure of adaptive (i.e., 'resilient') psychosocial functioning. To examine the neuro-immune responses to stress and their relationship with the brain and social environment, we will use a well validated functional magnetic resonance imaging (fMRI) task; the Montreal imaging stress task (MIST), and venepuncture. We will run group or dimensional comparisons in multiple levels of biological and psychological outcomes, as well as mediation and moderation analyses to study how key biological systems (i.e., the hypothalamic-pituitary-adrenal axis and the immune system) interrelate and interact with brain function and social influences in order to facilitate resilient functioning after CA. We hypothesize that resilient functioning will be facilitated by reduced morning cortisol and cytokine levels before and after the stressor and improved neural responses to such stress, as well as increased gray matter volume in the hippocampus and prefrontal cortex, enhanced inhibitory control and emotion regulation, and more friendship and family support. **Ethics and Dissemination:** This study has been reviewed and given favourable opinion by the National Research Ethics Service, NRES Committee East of England – Cambridge Central and external reviewers from the Royal Society (RGF\R1\180064 and RGF\EA\180029). The results of the RAISE study will be disseminated through (1) publications in scientific peer reviewed journals, (2) presentations on relevant scientific conferences and meetings, (3) publications and presentations for the general public and (4) through social media.

Keywords: Resilience; Stress; Childhood adversity; Maltreatment; Adolescence.

Strengths and limitations of this study

The RAISE study will provide a comprehensive evaluation of the neurobiological mechanisms that contribute to adolescent resilience.

We will use standardised and validated instruments of psychological functioning, childhood adversity, cognitive tasks, venepuncture and neuroimaging.

The exclusion of psychiatric patients will restraint the data to the resilience side of the spectrum

Child adversity will be assessed from self-reports that are subjected to reporting biases.

A longer recruitment period may be required due to Covid-19.

1. INTRODUCTION

Childhood adversity is the leading preventable risk factor for mental illness and substance abuse [1–8]. This kind of experiences, which can happen within the family environment (e.g., in the form of childhood maltreatment and/or intra-family adversity) or outside the household (e.g., trauma and bullying), can have a detrimental impact on a wide range of functions. For example, CA has been associated with physical (e.g., failure to thrive, poor adult health, and high mortality), cognitive (e.g., impaired inhibitory control and emotion regulation), and personal and interpersonal problems (e.g., negative self-cognitions, suicidal behaviours, increased peer rejection, social withdrawal, sexual maladjustment, aggression, and criminality) [8–16].

Importantly, adolescents with CA are at increased risk for and are more sensitive to psychosocial stress. In response to acute stress, the body reacts by releasing pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) [17]. These cytokines play a key role in stress reactivity and stress recovery [18]. Specifically, pro-inflammatory cytokines stimulate the hypothalamic-pituitary-adrenal (HPA) axis to release glucocorticoid hormones such as cortisol. Glucocorticoids, in turn, suppress the further release of cytokines from the immune system [19]. Thus, cortisol is an important anti-inflammatory compound in the body that is crucial for stress recovery. Pro-inflammatory cytokines and chemokines can cross the blood-brain barrier and negatively impact the function of brain regions involved in threat, reward, and executive functioning [20,21]. Indeed, acute stress has been associated with increased levels of pro-inflammatory cytokines in the amygdala and decreased pro-inflammatory cytokines in the medial prefrontal cortex (MPFC) [22]; regions associated with executive functions and emotion regulation [23–28]. Therefore, it is plausible that the alteration of these processes, or the inability to properly value and manage emotions, can lead to anxiety and/or depression in situations of negative affect [29–31].

However, although CA is associated with considerably lowered odds of adequate mental and physical health functioning later in life, a significant proportion of individuals with a history of CA function ‘better than expected,’ or, in other words, are “functioning resiliently” [32]. These individuals may have benefited from protective ‘resilience factors’ [33,34] which exist across social, cognitive, neuronal, physiological

and genetic levels. For example, good mental health after CA has been associated with increased hippocampal volume and greater connectivity between the central executive network and limbic regions as well as a greater ability to regulate emotions [35], higher self-esteem [36,37] and social support [32,38].

2. OBJECTIVES AND HYPOTHESIS

Resilient functioning has been associated with various components, ranging from genes and cellular mechanisms to higher-order biological systems and the social environment (see reviews in [39–41]; Figure 1). However, it is yet unknown whether and how neuro-immune responses to psychosocial stress differ in resilient vs. vulnerable adolescents with a history of CA. In this study, we aim to test the factors and mechanisms that facilitate resilient functioning, the interactions between those factors, and how they explain resilient responses to future stress. Specifically, we will address resilience by investigating how key biological systems (i.e., the HPA axis and the immune system) interrelate and interact with brain structure, brain function, and social influences to facilitate resilient functioning after CA.

All included participants will complete an online assessment to assess psychological functioning and early life experiences, an in-unit assessment day to assess neuro-immune and cognitive responses to stress, and an online follow-up assessment to assess psychological functioning after stress exposure. Resilient functioning will be quantified as the degree to which an individual functions better or worse than expected given their self-reported childhood adversity experiences ([32,39]; Figure 2). Specifically, to examine the neuro-immune responses to stress and their relationship with brain structure and function and the social environment, we will use a well-validated functional magnetic resonance imaging (fMRI) task, The Montreal imaging stress task (MIST), and venepuncture. Since stress increases circulating inflammatory protein levels in the blood, and high levels of inflammation predict later mental health disorders, we will examine whether resilience is related to lower levels of inflammation in response to psychosocial stress, and whether this is explained by improved brain responses to stress.

We hypothesise that resilient functioning will be facilitated by**:**

1) Reduced morning cortisol and cytokine levels before and after the stressor (i.e., a

latent factor constructed by serum high-sensitivity c-reactive protein (hs-CRP), TNF-α, IL-6 and Interleukin-1 factor beta (IL1β) levels, as these have been shown to be increased in those with CA and mental illness (i.e., [42–46]).

2) Reduced stress-related brain responses (i.e., amygdala, insula) and increased modulatory responses in the MPFC and anterior cingulate cortex (ACC) [47–49] during the MIST.
3) Increased functional connectivity between the DMPFC and emotion processing regions (e.g., insula, amygdala and hippocampus) during the MIST [50].
4) Increased gray matter volume in the hippocampus and prefrontal cortex [35,51–57].
5) Enhanced cognitive and emotional executive performance in behavioural tasks of inhibitory control and emotion regulation [29].
6) Higher self-esteem and more friendship and family support which will aid lower anxiety and perceived stress after the MIST [36,58].
7) Finally, we expect that the neurocognitive mechanisms that facilitate resilient functioning to stress at in-unit assessment will be related to improved cognitive and emotional functioning at follow-up (lower rumination, lower interpersonal stress, improved mood) in line with the neuro-immune network hypothesis ([21]; Figure 3).

3. METHODS

3.1. Recruitment and eligibility

Recent research suggests that the link between CA and immune markers in the blood are small for hsCRP immune biomarkers in adults with depression (hsCRP r=0.15, [59]). However, there are no studies investigating immune biomarkers in response to stress in adolescents. Therefore, it is difficult to determine the necessary sample size for our study. For instance, sample sizes for an intended power of 80% are r=0.15: n=345; r=0.2: n=193; r=0.3: n=84. Furthermore, it is well-established that sample correlations show fluctuations and are unstable in smaller samples. Simulation studies show that for a stable (i.e., replicable and generalizable) correlation, any sample would need to approach N=250 individuals [60]. For this reason, to increase power to find small effects, we will recruit N=200 participants. With this sample size, our study is appropriately powered to detect correlations from r>0.19.

Participants will be recruited from the general population and from previous studies conducted in the Department of Psychiatry of the University of Cambridge. First, we will contact participants from the Neuroscience in Psychiatry Network (NSPN) study who agreed to be contacted again. NSPN is a multi-centre accelerated longitudinal community cohort study (N=2389) focusing on normative adolescent to young adult development (between the ages of 14 and 24). Overall, this sample can be described as healthy, reporting low levels of psychopathological symptoms, behaviours and/or personality traits, and average mental wellbeing scores. From this cohort, we will contact those who agreed to be contacted for future research of whom family adversity scores were in the highest 25% of the entire NSPN cohort (≥75%=597 eligible individuals) as assessed by van Harmelen et al., 2017 [32]. Family adversity scores in NSPN were calculated using a Principal Component Analysis (PCA) on standard-normally transformed sum scores for the Measure of Parenting Style (MOPS) and the Alabama Parenting Questionnaire (APQ). Eligible participants will be contacted via email first. This email will include the participant information sheet (PIS) of the study. A reminder email will be sent after a month. Finally, we will contact those participants who cannot be reached by email via phone call. To recruit participants from the general population we will distribute flyers and advertisements in colleges, Addenbrooke's hospital, and online. Individuals expressing an interest in the study could either email or telephone a member of the research team and leave their contact details. A member of the RAISE study research team will then phone interested individuals. During the telephone call a member of the research team will discuss the content of the PIS and assess the inclusion and exclusion criteria in order to ensure they are eligible and fully aware of the nature of the study. Eligible participants will be emailed the PIS of the study. Potential participants will be given the opportunity to raise any queries regarding any aspect of the study including confidentiality, anonymity, storage and use of data, as well as the right to withdraw.

The inclusion criteria will be: aged 16-26 years old; able and willing to give informed consent; able to speak, write, and understand English; BMI between 18.5 and 29.9 kg/m2; have experienced adverse life experiences and/or CA within the family environment including childhood maltreatment (e.g., emotional, sexual and/or physical abuse, emotional and/or physical neglect) and intra-family adversity (e.g., marital distress/conflict, parental mental health problems and/or parental alcohol dependence,

violence and/or aggressive behaviour) before the age of 16; and willing to abstain from strenuous exercise for 72 hours prior to the in-unit assessment.

The exclusion criteria will be: alcohol or substance use disorder within the past 6 months; current disorders likely to compromise the interpretation of the data (including, but not limited to, psychiatric disorders, immunological disorders, cardiovascular disorders, endocrine and autoimmune disorders, malignancies or infections, or any other condition to be determined by the principal investigator or delegate); current medication likely to compromise the interpretation of immunological data (including, but not limited to, corticosteroids or any other substance to be determined by the principal investigator or delegate); and contraindications to MRI (e.g., pacemaker or other implantable device or pregnancy).

3.2. Testing protocol and procedure

All included participants will be asked to complete 3 phases:

Phase I: an online assessment to assess psychological functioning and early life experiences; phase II: an in-unit assessment to assess neuro-immune and cognitive responses to stress, and phase III: an online follow-up assessment to assess psychological functioning after stress exposure. Please see Figure 4 and sections below for a description of the measures included in each phase.

Phase I: online assessment

The online assessment will include signing an informed consent form (ICF), the completion of a set of self-report questionnaires and two cognitive tasks online. The self-report questionnaires included in this assessment will be the Mood and Feelings Questionnaire (MFQ; [61]), Revised Children's Manifest Anxiety Scale (RCMAS; [62]), Leyton Obsessional Inventory (LOI; [63]), the 10-item version of the Kessler Psychological Distress Scale (K10; [64]), the Child Behaviours Checklist (CBC; [65]), the Warwick-Edinburgh Mental Well-being Scale (WEMWBS; [66]), the Measure of Parenting Style (MOPS; [67]), the Alabama Parenting Questionnaire (APQ; [68]), the Cambridge Friendship Questionnaire (CFQ), Family Assessment Device (FAD; [69,70]), Rosenberg Self-Esteem Scale (RSES; [71]), Childhood Trauma Questionnaire (CTQ; [72]) and the Drugs, Alcohol, and Self-Injury Inventory (DASI; [73]). These

questionnaires will be used to calculate a composite measure of resilient functioning as described below. The cognitive tasks will be the Emotional Stroop task [74] and Emotional Regulation task [75]. More information about these measures is provided in the Supplementary Material.

Phase II: in-unit assessment

Following completion of the online assessment, participants will be contacted to schedule an appointment at Addenbrooke's Hospital (Cambridge, UK), after which they will receive a letter with the appointment details. This letter will include clothing and make-up regulations for brain scanning, time and location of the facilities where the evaluations will take place, as well as the research team's contact details. It will also reiterate that participation is voluntary and that participants can withdraw at any given time during the study. The second assessment will have a duration of 5 hours and will include (1) the completion of a second ICF, (2) a clinical evaluation, (3) a research nurse protocol, (4) an MRI session, and (5) the completion of a second set of self-report questionnaires. Please see the sections below for a description of the instruments included in each assessment.

Clinical evaluation

Participants will be asked to attend the clinical research facility (CRF) at Addenbrooke's Hospital at 9am, where they will be given a detailed overview of the study, asked to sign the ICF, and receive a low-fat breakfast adapted to their nutritional needs. The breakfast provided will exclude antioxidants such as glutathione and vitamins E and C to reduce the effects of these variables on the inflammatory markers addressed [76]. Subsequently, the clinical evaluation will take place. This session will include the evaluation of psychiatric disorders, handedness, physical activity, sleep and eating patterns, medications (e.g., oral contraception), and intellectual functioning. Specifically, we will use the following measures: The Mini-International Neuropsychiatric Interview (MINI; [77]), The Edinburgh handedness inventory [78], The Short-form Frequency Food questionnaire (SFFFQ; [79]) and The Wechsler Abbreviated Scale of Intelligence (WASI; [80]). The measures used will be supplemented with an interview. See the Supplementary Material for a description of the instruments used in this evaluation.

Physiological evaluation protocol

The clinical assessment will be followed by a physiological evaluation protocol. A research nurse will assess physical variables such as body temperature, height, weight, waist circumference, blood pressure (systolic and diastolic), and will implant a cannula to take blood samples during the assessment day.

Venepuncture

The implantation of the cannula will be conducted according to the standard Cambridge Clinical Research Facility protocol with risk protocols in place. These procedures include a brief interview about adverse experiences with blood assessment (such as fainting), as well as their preference for one arm or the other and if they want to lie down, or sit upright during the implantation of the cannula. Up to 30 mL of venous blood will be collected per participant for the measurement of cortisol, blood cytokines and immunophenotyping (i.e., basic immune cell counts and cell phenotyping). Blood will be extracted using an intravenous catheter inserted in the antecubital vein of the arm of the participants. 30 minutes following catheter insertion, the participants will undergo MRI scanning and will perform the psychological stress task (i.e., MIST). 1.2 mL K2 EDTA tubes will be taken for the analysis of immunophenotyping, 2.6 mL serum white tap tubes for the analysis of cytokines and 4.6 mL serum brown tap tubes for the analysis of cortisol. Bloods will be acquired at 4 time points: (-T1) 45 min before the start of the task (baseline line), (T0) right before the start of the task, (T30) right after the end of the task (peak cortisol) (T80) 80 min after the start of the task (delayed immune reactions) [81]. Please see Figure 5 for a representation of the venepuncture protocol.

Neuroimaging protocol

Before the scan, participants will complete an MRI screening form, the State-Trait Anxiety Inventory [82], and practice the MIST. Each participant will be in the MRI scanner for about 50 minutes. The MRI scanning session will comprise the following MRI sequences:

T1-weighted three-dimensional magnetisation-prepared rapid gradient-echo (MPRAGE) (6 mins). High spatial resolution T1-weighted structural scans will be used

to aid normalisation and visualisation of each of the other MRI modalities (as described below) and analyse brain structure (i.e., cortical thickness, grey matter volume).

Resting state functional MRI (7 mins). A resting state fMRI will be used to investigate effects of inflammation on brain functional connectivity.

Montreal Imaging Stress Task (30 mins). We will use a modified version of the Montreal imaging stress task (MIST; [83]). This task comprises a series of computerized mental arithmetic tasks with an induced failure component. The protocol consists of a training session conducted outside the imaging unit, and a test session during which the functional images are acquired. Please see the Supplementary Material for a description of the paradigm.

After the MRI session, participants will be debriefed. We will tell them that the task was designed to be impossible to accomplish and that it did not truly assess their ability to perform mental arithmetic. Then, we will ask them to complete the STAI-State, MFQ, Life Events Questionnaire [84], and any questionnaire from the online assessment that is incomplete. After completion of the post-MRI session, the participants will have a standard meal and be given time to relax. In addition, we will have a protocol for debriefing, an information letter with relevant types of support available, and a distress protocol in case the participant reports severe distress.

Phase III: follow-up online assessment

The follow-up and final assessment will be completed online within a month from the in-unit assessment and will include the third and last online ICF, the MFQ, RCMAS, and LEQ, as well as the following measures described in the Supplementary Material: Perceived Stress Scale (PSS; [85]), Interpersonal Sensitivity Measure (IPSM; [86]) and the 10-item Ruminative Response Scale (RRS-10; [87]).

Please see the Supplementary Material for a relation of the main risk and ethical issues associated with this protocol.

3.3. Patient and Public Involvement

A group of 3 adolescents participated in a Lived Experience Advisory group to assess the protocol and materials included in the study (e.g. Participant Information Sheet,

consent forms, questionnaires etc.). We have made the following changes as a result of their feedback: (1) we have included a risk protocol in case a participant feel distressed during the completion of the questionnaires, (2) we have increased the payment for the completion of the study to account for the time burden of the questionnaires and the distress associated with the cannulation, and (3) we have modified the participant information sheet in accordance with their suggestions.

4. ANALYSES

Clinical, questionnaire, and immunological data will be descriptively summarised. The significance threshold will be set at $p<0.05$ and Family-Wise Error (FWE) corrections will be applied to correct for multiple comparisons.

4.1. Preprocessing

Quantification of resilient functioning

Using the data collected during the first online assessment, we will calculate gender and age-related degree of resilient functioning based on the model described in van Harmelen et al. [32]; see also Ioannidis et al. [39] for a description of the benefits and pitfalls of this method. Specifically, we will conduct two PCAs; one for psychosocial functioning using standard-normally transformed individual total scores on the MFQ, RCMAS, LOI, CBC, K10 and WEMWBS; and another one for CA, including standard-normally transformed sum scores for the MOPS, APQ, FAD and CTQ. From both analyses, we will extract individual scores for the first component to reflect individual current psychosocial functioning and recalled CA experience scores. Next, we will regress the psychosocial functioning component score against the CA score, testing for possible linear, quadratic or cubic relationships. From this model, we will extract the residual scores as a measure of individual degree of resilient functioning: the extent to which an individual has better, or worse, psychosocial functioning than the average score expected given their CA experiences. For parsimony, we will refer to this as degree of 'resilient functioning' with higher scores reflecting better (conditional) psychosocial functioning outcomes. These individual resilient functioning scores will be utilised in the analyses described below.

Imaging preprocessing

Task-evoked and resting state fMRI data will be preprocessed using Statistical Parametric Mapping 12 (SPM12) (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and the CONN toolbox (https://web.conn-toolbox.org/) implemented in MatLab R2019b (Mathworks, Natick, MA, USA). Images will be corrected for movement artefacts, coregistered with the T1-weighted images, normalised to a standard EPI template in the Montreal Neurological Institute (MNI) space and spatially smoothed with an 8 mm FWHM Gaussian kernel. Quality control will be performed after each pre-processing step. Structural MRI will be analysed using the FreeSurfer image analysis suite, which is widely documented and freely available online (http://surfer.nmr.mgh.harvard.edu/).

Cortisol and immune markers preprocessing

Blood samples will be processed at the Core Biochemical Assay Laboratory (CBAL) (blood cytokines), Pathology (cortisol), and Immunology laboratory (immunophenotyping) at Addenbrookes Hospital (Cambridge, UK). 1ml of blood will be diluted 1:1 with Phosphate Buffered Saline (PBS) and stimulated with lipopolysaccharide (LPS) (LPS challenge) to analyse the production of IL-6 and other cytokines difficult to detect [88,89]. LPS-stimulated IL-6 and IL-1β production in peripheral leukocytes will be processed following a protocol originally developed by DeRijk et al. [90]. All cytokines, including serum TNF-α and hs-CRP will be measured using the MSD platform.

Phenotyping

50-100 ul of blood will be used for the enumeration of the major populations of immune cells (monocytes, granulocytes, NK cells, NKT cells, CD4+ and CD8+ T cells, and B cells). We will use multi-colour flow cytometry to count and analyse the size, shape, and properties of individual cells within heterogeneous populations. We will use multivariate methods (partial least squares and PCA) to reduce dimensionality and define populations of differentially co-expressed cell counts and the unbiased gating algorithm SPADE (Spanning-tree Progression Analysis of Density-normalized Events). SPADE generates an immune cell hierarchy by clustering phenotypically similar cells into groups which can be enumerated.

4.2. Statistical analyses

Age, gender, socioeconomic status, and education level will be included as covariates in all analyses. Additionally, for the imaging analysis we will include the total volume of grey matter (for the analyses of grey matter), a high-pass filter uses to remove low-frequency drifts in the data (for the analysis of fMRI data), and the signal fluctuations in white matter and cerebrospinal fluid and the subject-specific 6 realignment parameters and their first order derivatives (for the analyses of functional connectivity). Finally, phase of menstrual cycle, BMI, and tobacco smoking will be used in the analyses involving immune/cortisol markers.

Hypothesis 1: Individuals with higher resilient functioning will display lower baseline cortisol and blood cytokines, faster habituation, and less cortisol volatility:

We will use a PCA to derive a factor score for endocrine or inflammatory markers at the different time points. We expect there will be two components: one relating to baseline cortisol (or index of immune biomarkers), and one relating to cortisol *change* (or index of immune responsivity; see [91] for a similar approach). We will then utilise area under curve (AUC) analyses to examine whether degree of resilient functioning is more strongly associated with general immune status (i.e., the baseline cortisol measure) or cortisol responsivity (i.e., area under the curve with respect to ground (AUC_G) and area under the curve with respect to increase (AUC_I) (see [92] for specifics). We will validate this result using a simple multivariate regression to examine whether the two components of immune functioning independently predict resilient functioning outcomes.

Hypotheses 2-4: Higher resilient functioning is associated with more balanced and integrated neural systems:

For the event-related fMRI analysis, we will use both whole-brain and regions of interest (ROIs) approaches. To examine the effect of stress, we will examine brain responses to the contrast ‘experimental + control condition’ vs. ‘rest condition’. Then, in a second level analysis, we will conduct a multiple regression analysis with resilience scores as regressor of interest. The ROIs use will be the MPFC, ACC, amygdala, insula, and hippocampus. Finally, we will run a psychophysiological interaction (PPI) analysis to test hypothesis 3 (i.e., increased functional connectivity between the DMPFC and

emotion processing regions during the MIST).

For the analysis of resting state fMRI, we will use whole-brain and ROIs approaches. Our first approach will be a data-driven analysis. We will use intrinsic connectivity contrast (ICC; [93]). The use of ICC does not require a priori selection of a seed region but instead objectively defines how well each voxel is connected to the rest of the brain. Following the calculation of a resting state ICC map for each participant, we will test associations between ICC maps and resilient functioning. Specifically, we will correlate resilient functioning with the voxel level ICC using a multiple regression analyses in SPM. Finally, we will calculate functional connectivity maps and network involvement of the regions found to be associated with resilient functioning.

The significance thresholds will be set at $p<0.05$ after family-wise error correction for multiple comparisons across the whole-brain (pFWE<0.05) or the voxels of the different regions of interest ROIs (i.e., using small volume correction [SVC] procedures [pFWE-SVC<0.05]).

Hypothesis 5 & 6: Individuals with higher resilient functioning will display better cognitive control and greater levels of social support.

We will use Structural Equation Modelling (SEM) to examine the relations and interrelations described in hypotheses 5 & 6. Specifically, we hypothesise that, using a multiple indicators multiple causes (MIMIC) model, we will observe that individual differences in outcome (cognitive functioning) will be explained by partially independent and complementary neural systems (i.e., all paths shown will be significant when estimated simultaneously).

Hypothesis 7: Neurocognitive mechanisms that facilitate resilient functioning to stress are related to improved cognitive and emotional functioning at follow up.

Using mediation modelling (e.g. Figure 2), we will examine whether the mechanisms that facilitate resilient functioning to stress are related to improved cognitive and emotional functioning (i.e., rumination, interpersonal stress, mood, etc.) at follow up.

Immunophenotyping analyses

We will assess immunophenotyping data to determine whether or not resilient

adolescents can be distinguished in terms of immunophenotype levels. The proportion of each immune cell type will be used as the predictor variables in a Partial Least Squares (PLS) analysis, with resilient functioning as the response variable. Using this method, we hope to identify immune patterns that are associated with resilient functioning.

5. CURRENT STATUS

This study has been reviewed and given favourable opinion by the National Research Ethics Service, NRES Committee East of England – Cambridge Central and external reviewers from the Royal Society (RGF\R1\180064 and RGF\EA\180029). We are currently at year 3 of the project. As of March 2020, 102 participants have been recruited for the study. From those, 62 have completed phases I, II and II of the study. Only ten participants have withdrawn following informed consent due to various reasons (e.g. scheduling conflicts, presence of mental health disorders or found images presented in the Emotional Regulation Task overly distressing). Additionally, of the adolescents screened, the most common reasons for ineligibility are MRI incompatibility (e.g. dental braces), current psychotropic medication, and BMI outside 18-30. Due to the uncertainty caused by the COVID-19 outbreak we cannot anticipate when we will be able to finish the recruitment of our participants. However, we are approaching community organizations and agencies across Cambridge, including agencies working with victims of trauma to aid in the recruitment of participants with CA, and therefore we anticipate the finalization of the recruitment in three months from the time we start recruiting again.

6. DISCUSSION

The RAISE study aims to examine how resilient adolescents react to psychosocial stress in order to better understand the neurobiological mechanisms that contribute to adolescent resilience. We will examine how key biological systems (i.e., the HPA axis and the immune system) interrelate and interact with brain function and social influences in order to facilitate resilient functioning after CA. We hypothesize that resilient functioning would be facilitated by reduced baseline cortisol and cytokine levels before and after the stressor (i.e., a latent factor constructed by serum hs-CRP and TNF-α and IL-6 and IL1β levels) as these have been shown to be increased in those

with CA, mental illness, reduced stress related brain responses (i.e., amygdala, insula), and increased modulatory responses in the MPFC and ACC during the MIST. Moreover, we expect to find that the neurocognitive mechanisms that facilitate resilient functioning to stress during the in-unit assessment will be related to improved cognitive and emotional functioning at follow-up (lower rumination, lower interpersonal stress, improved mood). The findings from this study will help us to determine what sets resilient individuals apart on a neurobiological level. Such knowledge will be helpful to inform intervention strategies for individuals with a history of CA to prevent the development of mental health disorders, and ultimately increase resilience in individuals who have experienced adversity in early life. Although we will take a dimensional approach, examining individual variation in degree of resilient functioning and including individuals with past histories of psychiatric disorders and subthreshold mental health disorders, the exclusion of patients with current psychiatric disorders will limit the interpretability of the data and our findings. Future studies should include participants with current mental health disorders to ensure the representation of both dimensions of the spectrum.

DATA SHARING

Data will be available at the University of Cambridge Repository and accessed upon reasonable request.

AUTHOR CONTRIBUTIONS

Dr. Laura Moreno-López lead the recruitment and testing of participants and drafted the manuscript. Ms. Samantha N. Sallie, Konstantinos Ioannidis, Muzaffer Kaser, Katja Schueler, Adrian Dahl Askelund and Lorinda Turner were instrumental to the design of the study and reviewed the manuscript. The RAISE consortium members were or are instrumental to the setup and/or assessment of the RAISE study. Dr. Anne-Laura van Harmelen conceptualized and designed the study, drafted the original grant proposal, obtained financial support, oversaw all study procedures, and reviewed and revised this manuscript. All authors approved the final manuscript and agree to be accountable for all aspects of the work presented.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest associated with this project.

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FIGURE LEGENDS:

Figure 1. The complex neurobiology of resilience after childhood maltreatment (CM) [39]. Resilient functioning in those individuals who have experienced CM may be facilitated by larger prefrontal cortex (PFC) and hippocampal volume and connectivity, the ability to adequately regulate emotions and dampen stress responsivity, cortisol and proinflammatory baseline and responses, polygenic resilience effects, social support from the immediate environment, and the wider ecology. For readability, the location of the hippocampus is not correct. 5-HTTLPR serotonin-transporter-linked polymorphic region, ACTH adrenocorticotropic hormone; BDNF brain-derived neurotrophic factor, FKBP5 FK binding protein 5, IL-6 interleukin 6, MAOA monoamine oxidase A, mPFC medial PFC, NPY neuropeptide-Y, TNFα, tumour necrosis factor-α.

Figure 2. Risk to Resilient functioning in the NSPN sample of N=1980 adolescents [32]. Green and red lines indicate functioning that is better or 'resilient' (green) or worse 'vulnerable' (red) than expected. Psychosocial functioning reflects a factor score (mean=0, SD=1) derived from multiple measures of psychiatric symptomatology, personality traits, and mental wellbeing. CA reflects a factor score (mean=0, SD=1) from two measures which assess early life family experiences.

Figure 3. The neuro-immune network hypothesis of child maltreatment. Inflammatory protein and brain responses to psychosocial stress mediate the relationship between CA and psychological functioning.

Figure 4. Summary of the study protocol. Abbreviations: APQ=Alabama Parenting Questionnaire, CBC=Child Behaviours Checklist; CFQ=Cambridge Friendship Questionnaire, CTQ=Childhood Trauma Questionnaire, DASI=Drugs Alcohol and Self-Injury, EHI=Edinburgh Handedness Inventory, ERT=Emotion Regulation Task, FAD=Family Assessment Device, IPSM=Interpersonal Sensitivity Measure, K10=Kessler Psychological Distress Scale, LEQ=Life-Events Questionnaire, LOI=Leyton Obsessional Inventory, MFQ=Mood and Feelings Questionnaire, MINI=Mini-International Neuropsychiatric Interview, MIST=Montreal Imaging Stress Task, MOPS=Measure of Parenting Style, MPRAGE=T1-Weighted rapid three-dimensional radient-echo, PSS=Perceived Stress Scale, RCMAS=Revised Children's

Manifest Anxiety Scale, RRS-10=Ruminative Response Scale, RSES=Rosenberg Self-Esteem Scale, rs-fMRI=Resting state functional magnetic resonance imaging, SFFFQ=Short-Form Frequency Food Questionnaire, STAI=State-Trait Anxiety Inventory, WASI=Wechsler Abbreviated Scale of Intelligence, WEMWBS=Warwick-Edinburgh Mental Well-Being Scale.

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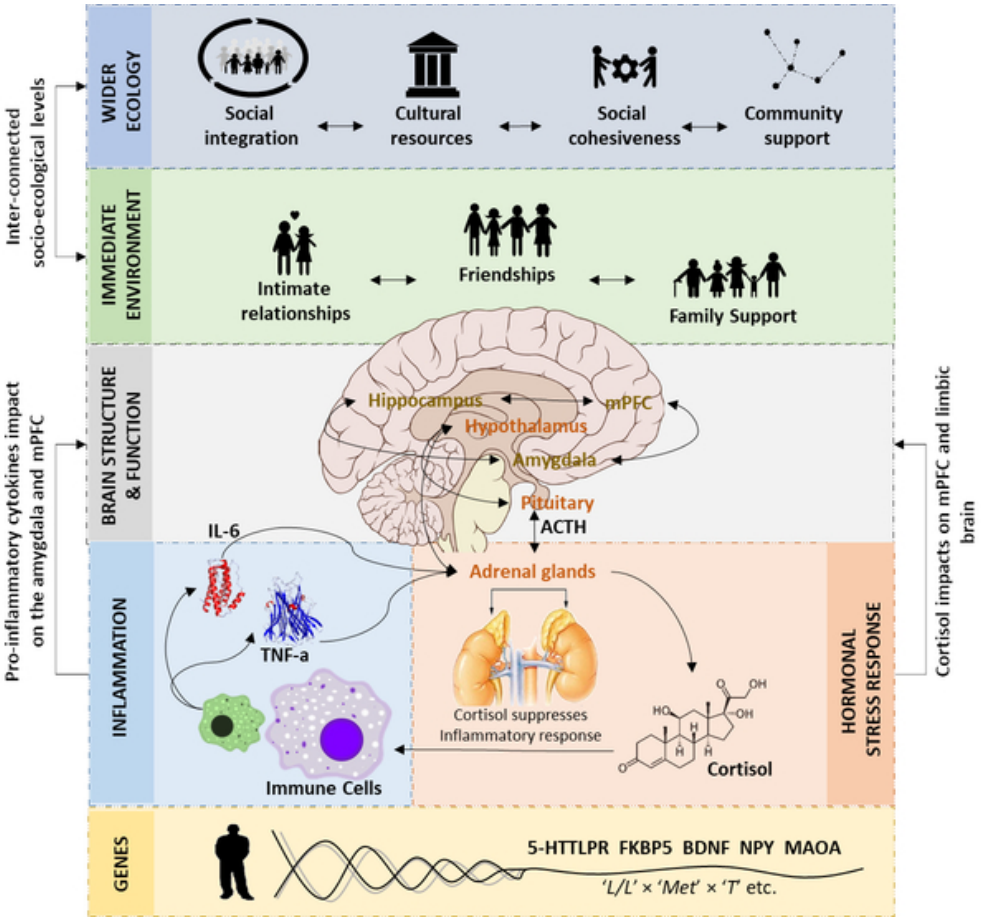

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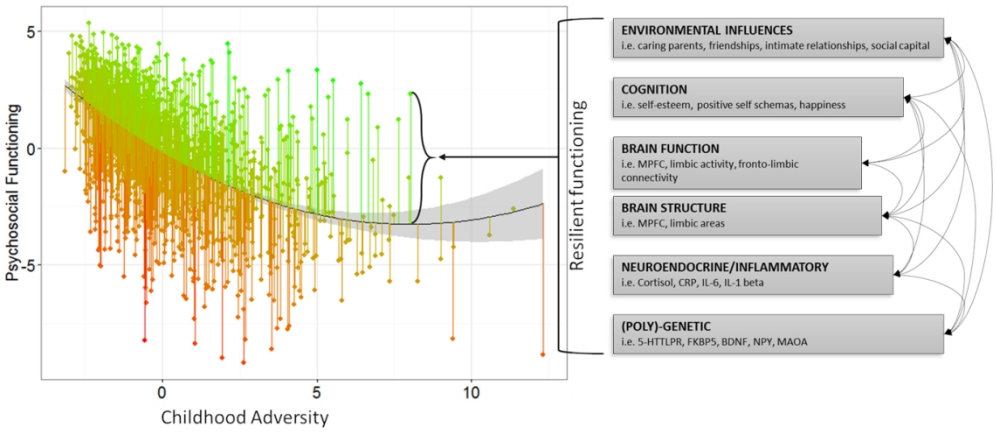

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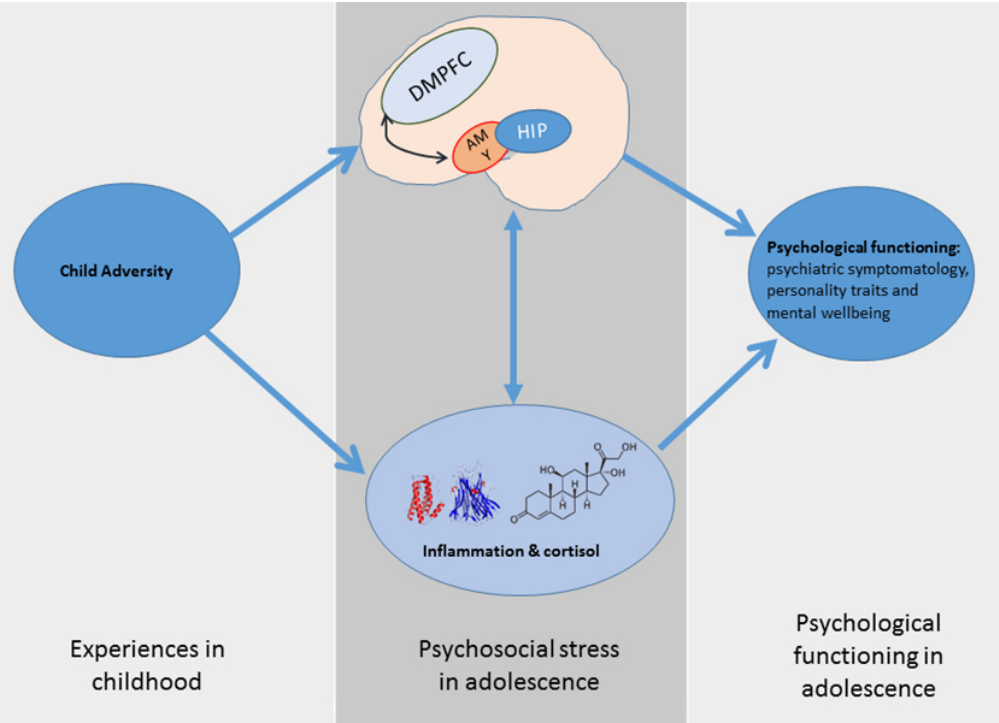

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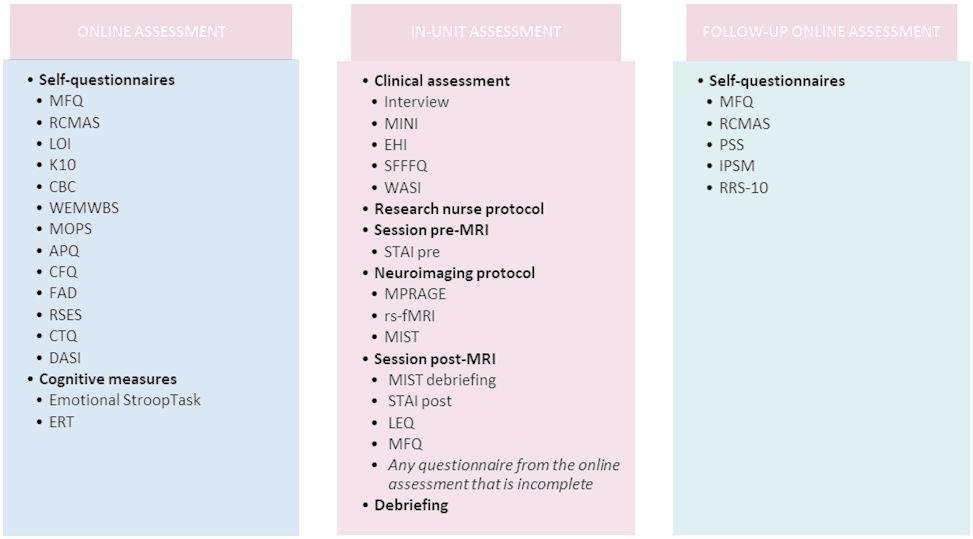

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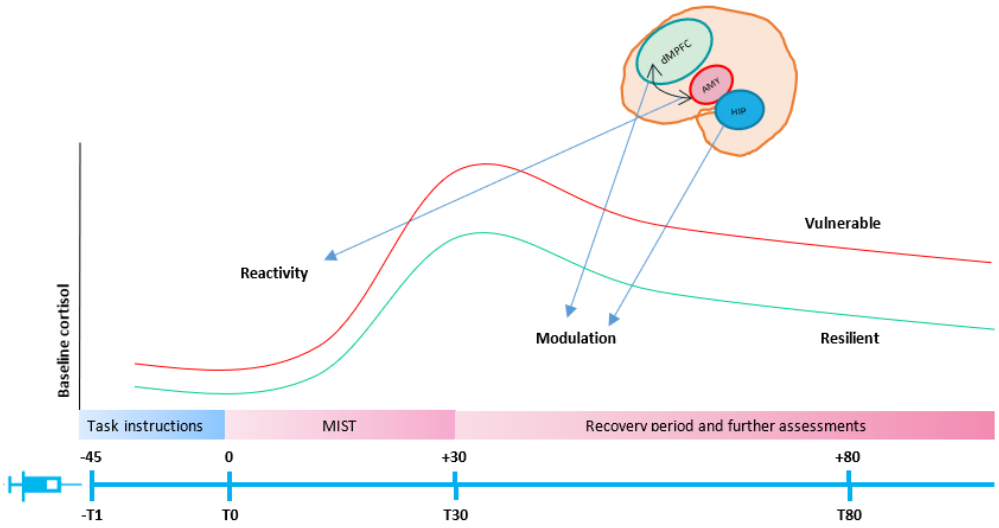

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Supplementary Material

The RAISE study protocol; a cross-sectional, multi-level, neurobiological study of studying resilience after individual stress exposure

Laura Moreno-López[1,*], Samantha N. Sallie[2], Konstantinos Ioannidis[3,4], Muzaffer Kaser[5,6], Katja Schueler[7], Adrian Dahl Askelund[8], Lorinda Turner[9], RAISE Consortium, & Anne-Laura van Harmelen[10,*]

Contents

Measures used in Phase I

Psychological functioning

Mood and Feelings Questionnaire. The Mood and Feelings Questionnaire (MFQ; Angold et al., 1995) is a 33-item instrument that was developed to measure depressive symptoms (over the course of two weeks prior and up to the date of the assessment) in children and adolescents from 8- to 18-year-olds. The MFQ has shown prognostic validity in clinical and non-clinical samples (Daviss et al., 2006; Wood et al., 1995). Higher sum scores indicate more symptoms.

Revised Children's Manifest Anxiety Scale. The Revised Children's Manifest Anxiety Scale (RCMAS; Reynolds & Richmond, 1978) is a 37-item questionnaire that includes three anxiety factors (physiological anxiety, worry/oversensitivity, and social concerns/concentration), as well as a total anxiety score. A higher score indicates a high level of anxiety.

Leyton Obsessional Inventory. The Leyton Obsessional Inventory (LOI; Bamber et al., 2002) is an 11-item questionnaire that measures obsessional/anxiety symptoms. Responses ranged from 'always,' 'mostly,' 'sometimes,' to 'never.' Higher scores indicate more obsessions.

Kessler Psychological Distress Scale. The 10-item version of the Kessler Psychological Distress Scale (K10; Kessler et al., 2002) measures frequency of nervousness, hopelessness, sadness, worthlessness, and fatigue. Responses are summed to create a total score, with higher scores signifying more psychological distress.

Child Behaviours checklist. The Child Behaviours Checklist (CBC) is an 11-item questionnaire for symptoms of antisocial behaviour based on the Diagnostic and Statistical Manual (DSM-IV) conduct disorder definition (Achenbach, 1991). Responses on these items ranged from 'always', 'mostly', 'sometimes' to 'never'. Internal consistency of the measure has been found to be good (Cronbach's $\alpha = 0.89$) (Rubio-Stipec et al., 1990). A high score indicates greater emotional and behavioural problems.

Warwick-Edinburgh Mental Well-being Scale. The Warwick-Edinburgh Mental Well-being Scale (WEMWBS; Tennant et al., 2007) was developed to enable the monitoring of mental wellbeing in the general population; and the evaluation of projects, programs, and policies aiming to improve mental wellbeing in the UK. It comprises 14 positively worded statements with five response categories from 'none of the time' to 'all of the time.' Higher scores indicate better mental well-being.

Cambridge Friendship Questionnaire. The Cambridge Friendship Questionnaire (CFQ) is an 8-item questionnaire assessing the number, availability, and quality of friendships. Higher scores indicate better perceived overall quality of friendships. The CFQ has good measurement invariance and external validity, and has demonstrated ecological validity across two samples (van Harmelen et al., 2016).

Family Assessment Device. The Family Assessment Device (FAD; Epstein et al., 1983; Miller et al., 1985) is a 12-item scale measuring overall functioning of the family. Six items describe healthy functioning and the other six describe unhealthy functioning. Each item is rated on a 4-point Likert scale (4='strongly agree,' 3='agree,' 2='disagree,' 1='strongly disagree'). The higher the score the worse the family functioning.

Rosenberg Self-Esteem Scale. The Rosenberg Self-Esteem Scale (RSES; Petersen, 1975) is a 10-item scale that measures positive and negative feelings of self-worth. All items are answered using a 4-point Likert scale format ranging from strongly agree to strongly disagree. Lower scores indicate lower self-esteem.

Childhood Trauma Questionnaire. The Childhood Trauma Questionnaire (CTQ; Bernstein et al., 1994) is a standardised, retrospective 28-item self-report inventory that measures the severity of different types of childhood trauma, producing five clinical subscales: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. Participants respond to each item in the context of 'when you were growing up' and answer according to a 5-point Likert scale ranging from 1='never' to 5='very often.' The score ranges from 5 to 25 for each subscale, with scores falling into four categories: none to low trauma exposure, low to moderate trauma exposure, moderate to severe trauma exposure, and severe to extreme trauma exposure. The measure also includes a 3-item minimisation/denial scale indicating the potential underreporting of maltreatment.

Drugs Alcohol and Self-injury. The drugs, alcohol, and self-injury inventory (DASI; Cassels et al., 2018) is a 14-item self-report measure of cigarette, alcohol, and drug use, and non-suicidal self-injury.

Child adversity

Measure of Parenting Style. The Measure of Parenting Style (MOPS; Parker et al., 1997) is a questionnaire designed to assess different approaches to parenting during the first 16 years of life. Participants have to evaluate each parent based on 15 statements (e.g., 'overprotective of me' and 'uncaring of me') using a four-point scale, yielding total scores for each parent on subscales labelled 'indifference,' 'abuse,' and 'over-control.' The sum scores of items in each category indicate the degree to which that parenting style was experienced by an individual.

The Alabama Parenting Questionnaire. The Alabama Parenting Questionnaire (APQ; Elgar et al., 2007) measures parenting practices. Participants are asked to rate how frequently each behaviour occurred in their family home on a 5-point scale ranging from 'never' to 'always.' High scores can indicate positive (i.e., involvement) or negative (i.e., inconsistent discipline, poor supervision, corporal punishment) parenting.

Cognitive functioning

Emotional Stroop task. In this task, participants have to categorise an adjective as angry, happy, or sad while ignoring the valence of the expression on a face upon which the adjective is superimposed (Preston & Stansfield, 2008). There are three blocks of trials: words only (32 trials), faces only (32 trials), and words and faces combined (64 trials). In the word-only block, the stimulus is one of the four emotion words (HAPPY, SAD, ANGRY, or SCARED; 8 trials each). In the face-only block, the stimulus is 1 of 16 pictures of facial affect (Ekman & Friesen, 1976), each appearing twice. The 16 pictures consist of 1 picture displaying each of the four emotions (happy, sad, angry, and scared) from each of four actors (older man, younger man, blonde woman, and brunette woman). In the word-and-face block, the stimulus is 1 of the same 16 faces with one of the four emotion words superimposed semi-transparently (89% transparent) over the face, centred vertically on the nose. On 48 trials in the word-and-face block, the facial expression matched the word (congruent trials—12 repetitions of each word matched to three iterations of each of the four actors expressing that emotion). On the other 16

trials, the facial expression did not match the word (incongruent trials—4 repetitions of each word matched at random to one of the possible remaining incongruent facial expressions). The emotional Stroop task generates two indices of affective executive control: the incongruency index and the congruency index. The incongruency index is the cost in reaction time to correctly categorize an emotional adjective when the background face depicts an incongruent emotional expression relative to when the face depicts a neutral emotional expression. The congruency index reflects the facilitation in reaction time to categorize an emotional adjective when the background face depicts a congruent facial expression relative to the neutral condition.

Emotion Regulation task. This task assesses the use of two strategies of cognitive emotion regulation: reappraisal and attentional control (i.e., distraction) (Kanske et al., 2011). Participants view neutral or negative emotional pictures on the screen one by one. After a short time, a text instruction appears on the screen to either 'View,' 'Reappraise,' or 'Distract' the emotion elicited by the current picture. Each picture appears in nearly every condition for approximately 6 seconds (in total, 128 trials). The 'View' condition refers to not regulating the emotional response to the picture at all. 'Reappraisal' means attenuating the initial emotional response by finding alternative interpretations of the picture. During 'Distraction' trials, a simple arithmetic task appears on the screen to which participants can then shift their attention in order to downregulate their emotions. After each trial, participants rate their current emotional state using the Self-Assessment Manikin (SAM). This task provides indices of individual general emotional reactivity, reappraisal effects and distraction effects. Scores are derived from rating differences between emotional categories and emotion regulation conditions.

Measures used in Phase II

Clinical evaluation

Mini-International Neuropsychiatric Interview. The Mini-International Neuropsychiatric Interview (MINI) was developed by Sheehan and Lecrubier (Sheehan et al., 1998) to meet the need for a brief, reliable, and valid structured diagnostic interview. The MINI contains 130 questions that assess 16 axis I DSM-IV disorders and is organised in diagnostic modules. For most modules, two to four screening questions

are used to rule out the diagnosis when answered negatively. Positive responses to screening questions are explored by further investigation of other diagnostic criteria (according to the standard MINI assessment protocol).

Edinburgh Handedness Inventory. The Edinburgh handedness inventory is an instrument used to assess the dominance of a person's hand in everyday activities (Oldfield, 1971). We will use the self-administered version.

Short-form Frequency Food Questionnaire. The Short-form Frequency Food questionnaire (SFFFQ; Cleghorn et al., 1016) is a measure of the quality of dietary habits. It evaluates the consume of 25 items including fruit, vegetables, fibre-rich foods, high fat and high-sugar foods, meat, meat products, and fish. The participants are asked to tick one option (ranging from 'rarely' to '5+ a day') for each of the items.

Wechsler Abbreviated Scale of Intelligence. The Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) is a short measure of IQ composed by four subtests: Vocabulary, Block Design, Similarities and Matrix Reasoning. We will use the subtests Vocabulary and Matrix reasoning to estimate the IQ of each participants.

Neuroimaging protocol

The State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983) is a measure of state and trait anxiety that includes items such as: 'I am tense,' 'I am worried,' 'I feel calm,' and 'I feel secure.' All items are rated on a 4-point scale (e.g., from 'Almost Never' to 'Almost Always'). Higher scores indicate greater anxiety. Internal consistency coefficients for the scale have ranged from 0.86 to 0.95.

Montreal Imaging Stress Task (30 mins). We will use a modified version of the Montreal imaging stress task (MIST; Dedovic et al., 2005). This task comprises a series of computerized mental arithmetic tasks with an induced failure component. The protocol consists of a training session conducted outside the imaging unit, and a test session during which the functional images are acquired. Please see the Supplementary Material for a description of the paradigm. The test session has two runs. Each run has three conditions: rest, control, and experimental. During the experimental session, the program is set to a time limit that is 10% less than the subject's average response time recorded during the training session. This approach induces a high failure rate. In

addition, the program continuously records the participant's average response time and the number of correct responses. If the participant answers a series of 3 consecutive mental arithmetic tasks correctly, the program reduces the time limit to 10% less than the average time for the 3 correctly solved tasks. Conversely, if the subject answers a series of 3 consecutive tasks incorrectly, the program increases the time limit for the following tasks by 10%. As such, under experimental conditions, a range of about 20% to 45% correct answers is enforced (Figure S1). Moreover, between experimental runs, the participant will see a screen with information about their performance, reminding them that the average performance is about 80%–90% correct answers and that they must improve their performance. During the control condition, mental arithmetic is presented with the same level of difficulty and at the same frequency as during the experimental sessions, but no time restriction is enforced, and individual performance and average users' performance are not displayed. Feedback (“correct” or “incorrect”) is still shown after each task, but because of the absence of a time limit, average performance increases to about 90%. Finally, during the rest condition, the interface of the computer program remains on the screen, but no tasks are shown. After the first run, a member of the radiographer team will show disappointment about the participant’s performance and ask him/her to try harder (e.g., “You are not doing as well as we had hoped”, “Please remember that your performance needs to be close to the average to allow us to use your data”, “Let’s try it again” etc.).

The Life-Events Questionnaire evaluates recent undesirable and desirable life events and friendships (LEQ; Goodyer et al., 1997). Participants are asked to rate 13 major life events during the preceding 12-month period that may have affected them. These may include changes in school, deaths, household disasters, friendship difficulties, and illnesses. Respondents are asked how they felt about the event on a scale of 1=‘very pleasant/happy’ to 5=‘very unpleasant/sad/painful.’ A quantitative estimate of the adverse life events is obtained by summarising the number of events rated either 4 or 5 for more than two weeks.

Measures used in Phase III

Perceived Stress Scale. The Perceived Stress Scale (PSS; Cohen et al., 1983) is the most widely used psychological instrument for measuring the perception of stress. This 10-item scale measures the degree to which situations in one’s life are appraised as

stressful. Items are designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes direct queries about current levels of experienced stress. Scores can range from 0-40 with higher scores indicating higher perceived stress.

Interpersonal Sensitivity Measure. The Interpersonal Sensitivity Measure (IPSM; Harb et al., 2002) is a 28-item scale that assesses excessive sensitivity to the interpersonal behaviour of others, social feedback, and (perceived or actual) negative evaluation by others. The measure includes a total score and three subscale scores: interpersonal worry and dependency (eleven items; e.g., 'I worry about what others think of me'), low self-esteem (ten items e.g., 'If other people knew what I am really like, they would think less of me'), and unassertive interpersonal behaviour (eight items; e.g., 'I find it hard to get angry with people').

Ruminative Response Scale. The 10-item Ruminative Response Scale (RRS-10; Treynor et al., 2003) is one of the most widely used self-report measures of rumination, comprising 10 items and describing the factors of brooding and reflection. Each item is rated on a 4-points Likert scale ranging from 1='never' to 4='always'). The total score ranges from 10 to 40, with higher scores indicating higher degrees of ruminative symptoms.

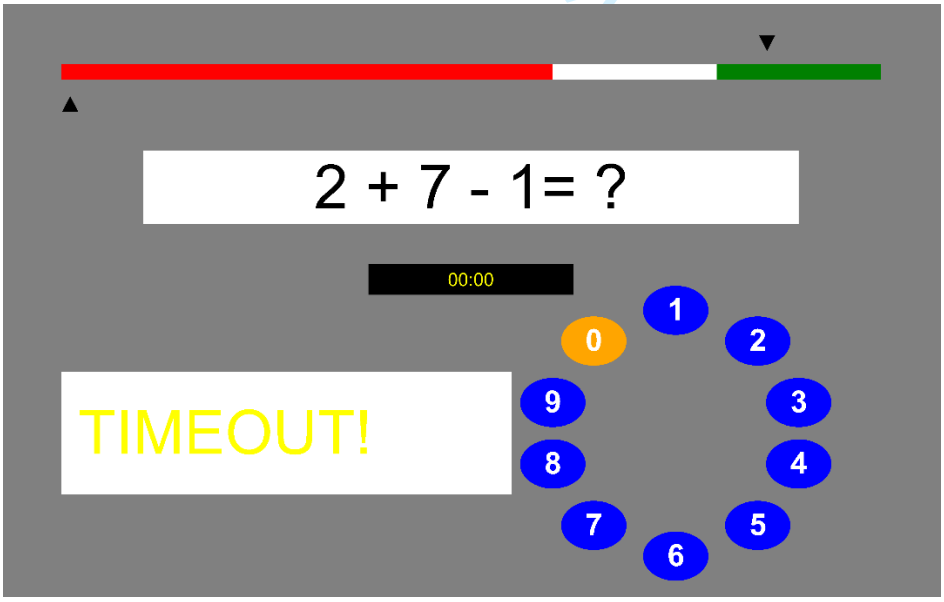

Figure S1. Graphical user interface of the MIST. From top to bottom, the figure shows the performance indicators (top arrow=average performance, bottom arrow=individual subject's performance), the mental arithmetic task, the progress bar reflecting the imposed time limit, the text field for feedback, and the rotary dial for the response submission.

Risk and ethical issues

The main risk and ethical issues associated with the protocol are: 1) Consent, 2) Confidentiality, 3) Risks and costs of participation, and 4) Compensation for participation.

Consent

Our participants will be 16 years old or older. We will obtain informed consent for each of the phases of the study: first, we will obtain consent verbally over the telephone when participants express their interest in the study. We will go through the PIS and inclusion/exclusion criteria to ensure participants are aware of the nature of the study. Participants will have opportunities to ask questions and details regarding confidentiality, anonymity, storage and use of data, as well as right to withdraw. Only those participants who meet the inclusion criteria and are willing to participate in the study will be invited to do so. We will send them an email with the links and instructions to complete the first online ICF, a set of self-rate questionnaires, and three cognitive tasks online. On the day of the scanning we will obtain informed consent in writing before the participants start the assessments. We will ensure that consent is voluntary and that participants are fully informed by asking whether they have understood everything on the form and whether they have any questions regarding the different assessments. We will then reiterate that participation is voluntary and that they can stop the evaluations at any time. Finally, and within a month of their in-unit evaluation at Addenbrooke's Hospital, we will send them an email with the link to complete the last online ICF and a set of self-rate questionnaires.

Confidentiality

Identifiable data will be linked by a unique ID number to the participant's anonymised study assessments. Participants will be carefully monitored to ensure that the procedures are followed in accordance with the UK Data Protection Act 2018. Only RAISE study team members, who are fully aware of their responsibilities to conform to the Data Protection Act 2018, will be allowed access to the personally identifiable data which will be stored in a highly secure, password encrypted database.

The transfer and storage of the participant's biological samples (venous blood) will be appropriately handled by our collaborators according to their standard operating procedures. All tissue samples will be labelled with a non-identifiable participant ID, date of birth, data of collection and sample identifier (i.e., serum), and stored in a -80° Celsius freezer at Addenbrookes Hospital. All samples will be scanned into a secure electronic database (i.e., RedCAP) to track their location and to ensure that all samples are accounted for.

All participants will be asked to provide consent to contact their parents (<18 year old participants) or general practitioner, in case the collected data suggest that the participant might require further clinical assessment or treatment.

Risks and costs of participation

Distress during the completion of the questionnaires and cognitive tasks online: some participants may find the questionnaires (e.g., CTQ, MFQ) or the cognitive tasks (e.g., emotion regulation task) distressing. In order to minimise this emotional burden we have made sure that both the questionnaires and cognitive tasks included in the study reiterate that the participant may withdraw at any time during the completion of the questionnaires/tasks, in which case, they will see a screen with a list of local mental health resources with activated web links. Moreover, following the completion of the Emotional Regulation Task, participants will be debriefed to inform them that the task was designed to produce an emotional response and it is completely normal and even intended that they reacted emotionally to the pictures presented.

Self-injury and suicidality disclosure: self-injury and suicidality disclosure will be addressed during the completion of the questionnaires online and at the end of the in-unit assessment. The completion of the questionnaires is configured in such a way that the RAISE study research team will receive an immediate email if the participant discloses current self-injury or suicidality (i.e., affirmative answer on Q19 of the MFQ and/or Qs11-12, or Qs14-15 of the DASI). The principal investigator and/or a suitable member of the research team will then review the participant's questionnaires with the psychiatrists affiliated with the study and if there is concern about imminent risk of self-injury or danger to the participant (e.g. disclosed current physical, sexual, or emotional abuse), they will call the participant. This will also be an opportunity to gather further

details (particularly regarding suicidal thoughts) and clarify whether they are in need of, and seeking, the appropriate help. If the risk is imminent, we will suggest the participant call First Response Service (111) or attend E&A. If the risk is not imminent and the participant is 18+ we will suggest they talk to their GP. If the participant is 16-17, we will suggest they talk to their GP and their parents/the people they live with about how they are feeling. If the participant refuses to seek help, we have a duty to care and it will be necessary to breach confidentiality. If the participant is 18+ we will contact their GP. If the participant is 16-17 we will contact their parents/guardians first. If contacting parents/guardians is contraindicated because of poor guardians-participant relationships (e.g., when disclosing to a parent may increase risk of suicide), direct contact with the participant's GP, or other local clinic or clinical support will be sought. Important points to note during disclosure to parents/guardians include: explaining that the study measures are not clinical instruments and thus cannot be used to detect future risk with absolute certainty, expressing concern about their child's responses to specific items, reinforcing that the safety of their child is of primary importance, helping them to think about how to get a psychological evaluation of their child and encouraging them to do so, reminding them that any information shared by their child was difficult for them to disclose, and recommending non-punitive and sensitive behaviour towards their child with regard to the issue. In the in-unit assessment, in addition to having lunch and time to relax, we will have a distress protocol in place in case the participant reports severe distress during or after the assessments.

Risks associated with the venepuncture: to minimise discomfort, blood taking will be conducted by a clinical research nurse, according to the standard Clinical Research Facilities operating procedures and risk protocols. The participant will have time to rest after the procedure.

Distress and discomfort during brain scanning: the MIST task is intended to be stressful. Therefore, after the session, we will debrief our participants by explaining to them that the task was designed to be impossible to accomplish and that it did not assess their true ability to perform mental arithmetic. Moreover, MRI scanners are very loud. In order to reduce any potential discomfort, all participants will be given earplugs for aural protection. MRI scanning also requires participants to lie still in the scanner which some may find uncomfortable or may induce feelings of claustrophobia. We will ensure that

participants are as comfortable as possible by providing neck and arm pillows. Furthermore, we will make sure that participants can communicate with a member of the research team at any time during scanning. They will be informed that if they would like to stop they may do so by pressing a button which will be easily accessible to them at all times.

Potential clinical findings: we will obtain consent from participants to inform their GP if any clinically relevant information comes to light during their participation in the study. In all cases, we will discuss it with a specialist and contact the participant first. If the participant refuses to seek help, we have a duty to care and it will be necessary to breach confidentiality. For neuroimaging findings, the Wolfe Brain Imaging Center (where the acquisition of the images will take place) policy is that all studies will include at least T1 and T2 weighted datasets, that are internally reported by a clinically qualified reviewer, who will refer to the WBIC clinical lead. The clinical lead will counsel the individual regarding further clinical referrals (including potential GP referral).

Time burden: the online assessments are not expected to last more than 2 and 1 hours respectively. During the in-unit assessment each participant will be tested for no longer than 5 hours in total. These durations will be clearly communicated to the participants at the beginning of the study.

Compensation for participation

To compensate for their time, participants will be paid £150. In addition, breakfast and lunch will be provided on the in-unit assessment day.

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