

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.....	2
Psychological functioning	2
Child adversity.....	4
Cognitive functioning	4
Measures used in Phase II	5
Clinical evaluation.....	5
Neuroimaging protocol.....	6
Measures used in Phase III.....	7
Risk and ethical issues.....	9
Consent.....	9
Confidentiality	9
Risks and cost of participation	10
Compensation for participation	12
References	13

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., 2016) 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.

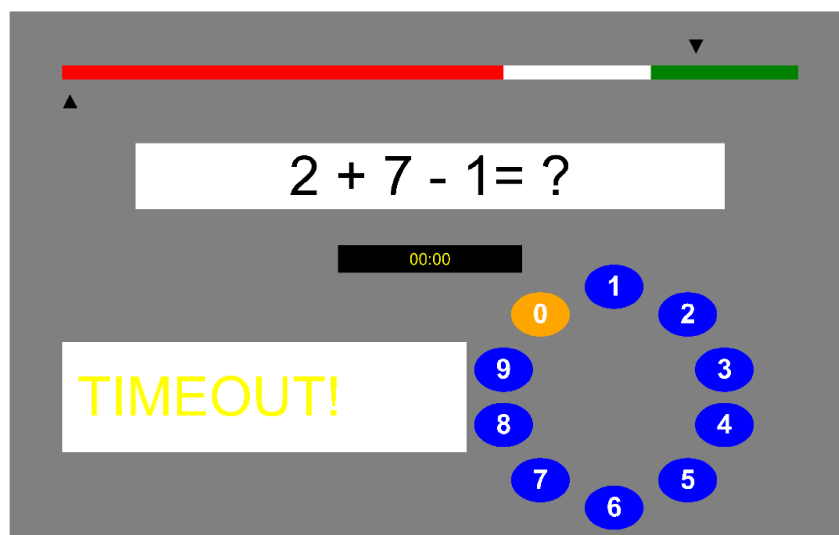


Figure S1. Graphical user interface of the MIST. From top to bottom, the figure shows the performance indicators (top arrow=average performance, bottom arrow=individual subject's performance), the mental arithmetic task, the progress bar reflecting the imposed time limit, the text field for feedback,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